MECHANISMS OF ENHANCED NON-VIRAL GENE DELIVERY TO HUMAN MESENCHYMAL STEM CELLS INDUCED BY GLUCOCORTICOID PRIMING

Andrew Hamann, Biological Systems Engineering, University of Nebraska-Lincoln
andrew.hamann@huskers.unl.edu
Kelly Broad, Biological Systems Engineering, University of Nebraska-Lincoln
Albert Nguyen, Biological Systems Engineering, University of Nebraska-Lincoln
Angela K. Pannier, Biological Systems Engineering, University of Nebraska-Lincoln

Key Words: hMSCs, transfection, glucocorticoid, priming, mechanisms

Background: Because of unique roles in wound healing, trophic tissue support, immunomodulation, differentiation ability, and immune privileged status, human mesenchymal stem cells (hMSCs), which can be easily derived from many adult tissues (e.g. bone marrow (BMSCs) and adipose tissue (AMSCs)), are under intense study for the applications of cell and gene therapeutics, as well as tissue engineering and regenerative medicine[1]. Genetic modification of hMSCs could allow for targeted delivery of transgenic therapeutic factors or genetically-guided differentiation. Non-viral gene delivery (i.e. cationic polymer- and lipid-mediated) is safer and more flexible than immunogenic and mutagenic viral vectors[2], but it is less effective, especially in hMSCs (i.e. maximum 10-30% transfection)[3]. As part of an approach to understand molecular mechanisms of non-viral gene delivery[4] and 'prime' cells to be more receptive to transfection[5], our lab recently demonstrated that transgene expression from lipofected hMSCs can be increased about 10-fold by priming cells, 30 mins before plasmid DNA (pDNA) transfection, with 100 nM dexamethasone (DEX), a glucocorticoid (Gc) drug, relative to EtOH vehicle control (VC)[6]. This work investigates the mechanisms by which Gc priming enhances non-viral gene delivery, which are currently unknown. Studies provide insights into the biological processes of Gc priming and transfection to inform future gene delivery technologies, and characterize a simple protocol to significantly enhance non-viral gene delivery of therapeutic transgenes for future clinical applications.

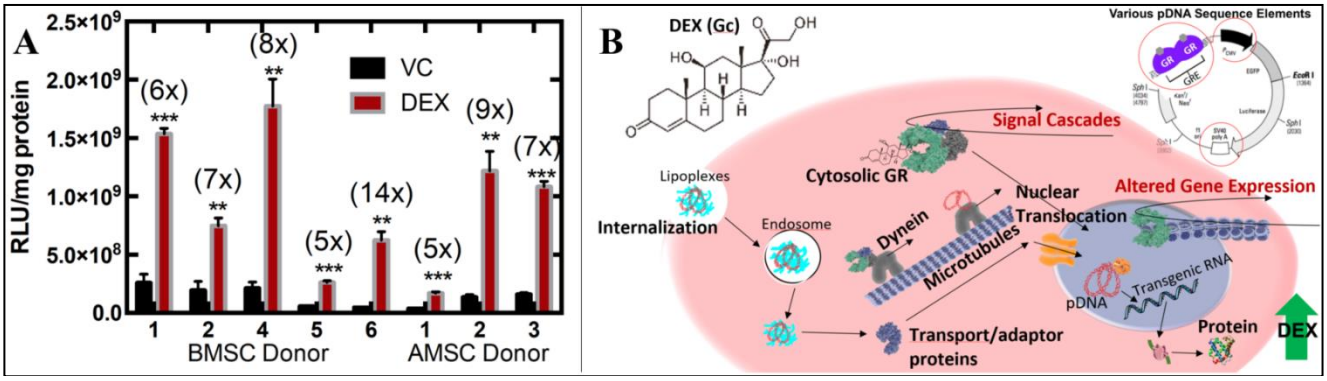


Figure 1-- A) BMSC and AMSC luciferase transgene expression treated with EtOH vehicle control (VC) or 150 nM DEX B) Summary of investigations into molecular mechanisms of hMSC transfection and DEX priming (e.g. pDNA internalization and intracellular transport, endogenous signaling and gene expression, pDNA sequence interactions, transgene transcription and translation).

Results & Discussion: DEX significantly increased transgene expression over VC in all BMSCs and AMSCs, derived from multiple human donors (i.e. 5- to 14-fold) (Fig. 1A). Enhanced non-viral gene delivery by Gc treatment was mediated by binding of the cytosolic glucocorticoid receptor (GR) (Fig. 1B), as treatment with other steroids resulted in no enhancement. Inhibiting nuclear import pathways dramatically decreased transgene expression in the absence of DEX, but did not decrease the fold-change enhancement by DEX priming. Cytoplasmic transport inhibition modestly decreased fold-change enhancement by DEX priming, possibly by inhibiting GR or pDNA trafficking. Enhancement by DEX treatment occurs regardless of any pDNA sequence element changes. DEX priming did not increase cellular or nuclear internalization of pDNA, or increase transgene RNA expression. DEX treatment modulated endogenous gene expression to increase hMSC total protein synthesis after transfection, contributing to increased transgenic protein production. Studying mechanisms of priming cells for transfection provides greater understanding of molecular parameters important to gene delivery and characterizes practical protocols to improve delivery of clinically-relevant transgenes.

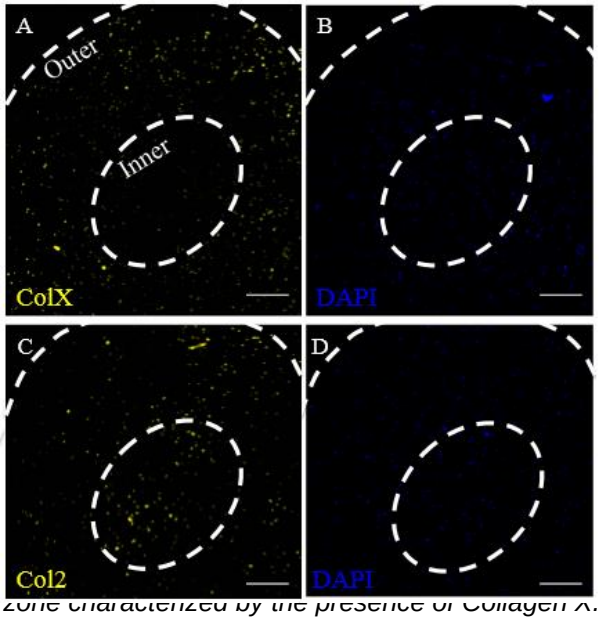
References: [1] D'Souza et al, BMC Med, 2015, 13, 186. [2] Nayerossadat et al, Adv Biomed Res, 2012, 1, 27. [3] Hoare et al, J Gene Med, 2010, 12, 207. [4] Plautz et al, Mol Ther, 19, 2144. [5] Nguyen et al, Bioeng Transl Med, 2016, 1, 123. [6] Kelly et al, Mol Ther, 2016, 24, 331.

USE OF A THREE-DIMENSIONAL IN VITRO ALGINATE HYDROGEL CULTURE MODEL TO DIRECT ZONAL FORMATION OF GROWTH PLATE CARTILAGE

Alek Erickson, Cell Biology, and Anatomy, University Nebraska Medical Center, Omaha, Nebraska
Sophia Walsh, Department of Biological Systems Engineering, University Nebraska Lincoln, Lincoln, Nebraska
Andrew Dudley, Cell Biology, and Anatomy, University Nebraska Medical Center, Omaha, Nebraska
Angela Pannier, Department of Biological Systems Engineering, University Nebraska Lincoln, Lincoln, Nebraska

Key Words: growth plate cartilage, IHH/PTHrP signaling, 3D culture model, tissue architecture, alginate hydrogel

Growth plate cartilage is found at the ends of long bones, and is responsible for the growth of the bones as a person is developing. The architecture of this growth plate is very specific and contributes to proper function to allow for bone growth. Although there are many factors known to be involved in the formation of the growth plate and its proper regulation, the exact mechanisms involved in these processes are not fully understood. So far, previous attempts to recapitulate a functioning growth plate *in vitro* have been unsuccessful. In this study, a new method to study the growth plate and the mechanisms involved in its formation was developed using an *in vitro* cell culture system made of alginate hydrogel scaffolds. Chondrocytes isolated from neonatal mouse growth plates were encapsulated within hydrogel beads and cultured. The addition of exogenous factors to the culture medium allowed the study of the parathyroid-related protein-Indian hedgehog (PTHrP-IHH) pathway, a known feedback loop necessary in the regulation of bone growth. Supplemental PTHrP increased proliferation and inhibited hypertrophy of encapsulated chondrocytes, while maintaining chondrocyte differentiation. The addition of IHH or thyroxine to bead cultures stimulated the formation of a hypertrophic zone located around the surface of the beads (Figure 1). This study demonstrates the ability of this culture system to induce proper zonal architecture of the growth plate, and has lead to further studies of the mechanisms involved in this zonal formation. Currently, the addition of extra-cellular matrix binding proteins to alginate scaffold, such as heparin or chondroitin sulfate, is being studied for its ability to retain growth factors and affect architecture formation through cell migration and signaling. Also, knockout mice and other soluble factors known to be involved in the PTHrP-IHH pathway, including smoothened and Wnt5a, are being used to study the specific mechanisms and pathways involved in zonal formation of the growth plate. The use of this culture model to elucidate all the necessary factors involved in the formation of a functioning growth plate will allow the advancement of regenerative medicine in the field of developmental cartilage damage and disease.



zone characterized by the presence of Collagen X.

DESIGNING AND UTILIZING SYNTHETIC EXTRACELLULAR MATRICES TO PROBE BREAST CANCER CELL ACTIVATION IN RESPONSE TO MICROENVIRONMENT CUES

April Kloxin, University of Delaware
akloxin@udel.edu
Lisa Sawicki, University of Delaware
Elisa Ovadia, University of Delaware
Lina Pradhan, University of Delaware

Key Words: hydrogel, ECM mimics, breast cancer, three-dimensional culture, controlled microenvironments

Interactions between breast cancer cells and their microenvironments are essential in tumor growth, metastasis, and recurrence. The tumor stroma undergoes constant structural changes, including degradation, redeposition, and crosslinking of collagens with gradients in matrix stiffness and composition that drive invasion and metastasis. At metastatic sites, similar remodeling events that occur with injury and aging are hypothesized to promote reactivation of dormant tumor cells in recurrence. Approaches are needed for testing hypotheses about pivotal cell-matrix interactions in the progression of breast cancer for identifying key regulators and improving treatment strategies.

In this work, we have designed well-defined materials to mimic key aspects of tumor microenvironments toward studying such complex phenomena *in vitro*. Specifically, we have created synthetic extracellular matrices with well-defined biophysical and biochemical properties that enable three-dimensional (3D) culture of breast cancer cells and niche cells over weeks. A biologically inert polymer, multi-arm poly(ethylene glycol) functionalized with thiols (M_n ~ 20 kDa), was reacted with integrin-binding and cell-degradable peptides decorated with one and two allyloxycarbonyl protecting groups, respectively, by rapid light-triggered thiol-ene polymerization for independent control of matrix density and composition. The elasticity, or 'stiffness', of these matrices has been tuned to mimic a variety of soft tissues (Young's modulus E~0.5-20 kPa), from healthy and cancerous mammary tissues to metastatic site bone marrow and lung tissues. Further, the biochemical content has been tuned with receptor-binding peptides derived from laminin (IKVAV, laminin receptor), collagen ($(POG)_3POGFOGER(POG)_4$, $\alpha_2\beta_1$ and $\alpha_1\beta_1$), and fibronectin/vitronectin (RGDS, $\alpha_V\beta_3$ and $\alpha_5\beta_1$ amongst others) and a crosslinking peptide derived from collagen (GPQG↓IWGQ, degraded MMP-1, -2, and -9 amongst others).

We hypothesized that a microenvironment rich in collagen and fibronectin/vitronectin, mimicking aspects of remodeled tissues, would activate breast cancer cells relative to a laminin-rich epithelium-like microenvironment, building upon seminal studies in naturally-derived matrices and *in vivo*. To test this, we cultured breast cancer cells of different metastatic potential (estrogen receptor positive [ER+, T47Ds] and triple negative [ER-, MDA-MB-231s]) within different matrix densities and compositions. Both cell types exhibited high viability (> 90%), and cell activation in response to different matrix compositions was assayed by examining proliferation (metabolic activity, Ki-67, cell/cluster number and volume) and phenotype (morphology; E-cadherin, vimentin). Increased matrix density decreased elongation of ER- cells and proliferation of both cell types. Increased collagen content increased the proliferation of the ER+ cells and proliferation and elongation of ER- with mass and stellate morphologies, respectively, like observed in naturally-derived matrices. These studies demonstrate a new tool for controlled 3D culture of breast cancer cells relevant for both fundamental and applied research, with on-going investigations incorporating niche cells and triggered matrix changes.

MICROFLUIDIC ACINI-ON-CHIP PLATFORMS AS A TOOL TO STUDY BACTERIAL LUNG EXPOSURE

Arbel Artzy-Schnirman, Department of Biomedical Engineering, Technion-Israel Institute of Technology, Haifa, Israel, arbel@bm.technion.ac.il
Patrick Carius, Helmholtz-Institute for Pharmaceutical Research Saarland (HIPS), Saarbrücken University, Germany
Shani Elias-Kirma, Department of Biomedical Engineering, Technion-Israel Institute of Technology, Haifa, Israel.
Nicole Schneider-Daum, Helmholtz-Institute for Pharmaceutical Research Saarland (HIPS), Saarbrücken University, Germany
Claus-Michael Lehr, Helmholtz-Institute for Pharmaceutical Research Saarland (HIPS), Saarbrücken University, Germany
Josué Sznitman, Department of Biomedical Engineering, Technion-Israel Institute of Technology, Haifa, Israel

Key Words: Acinus-on-chip, Epithelial barrier, Bacterial contamination, Cytokines secretion, Lipopolysaccharide

Bacterial invasion of the respiratory system leads to complex immune responses involving many cell types. In the alveolar regions, the first line of defense includes the alveolar epithelium, secreted surfactant, alveolar lining fluid and alveolar macrophages. The epithelium consists of alveolar type I and type II cells. Both cell types are known to have immuno-modulatory functions characterized by the secretion of pro-inflammatory cytokines. Epithelial *in vitro* models offer attractive platforms to investigate biological functionality, but have typically relied on traditional well plate assays that come short of mimicking the complexity of the airway environment and do not capture physiological flows or relevant anatomical features. In the last decade, microfluidics have gained significant momentum in laying the foundations for constructing *in vitro* models that mimic physiologically-relevant organ functions. Here we propose to use acinus-on-chip platforms that mimic more closely native acinar microflows at true scale in a multi-generation alveolated tree. Acinar chips are cultured with human Alveolar Epithelial Lentivirus immortalized (hAELVi) cells at an air-liquid interface (ALI); such cells show alveolar type I like characteristics and maintained barrier function, leading to high trans-epithelial electrical resistance (TEER) in analogy to primary cells harvested from human tissue. To model bacterial infection, i.e. a strong stimulator of the innate arm of the immune system, lipopolysaccharides (LPS) will be used. LPS is a major outer surface membrane protein expressed on Gram-negative bacteria. The alveolar epithelium is exposed to LPS-laden aerosols and cell response is monitored mainly by secretion of pro-inflammatory cytokines. Our acinus-on-chip allows quantitative on-line measurements of alveolar barrier function, absorption kinetics and immunologically relevant responses, giving further insight to the role played by type I alveolar cells in lung immunity.

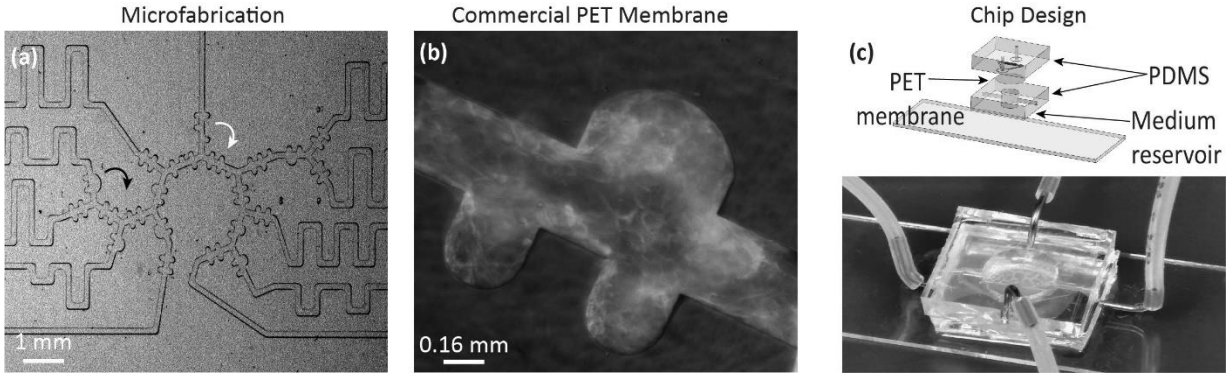


Figure 2 (a) PDMS-based model of developing acinar airways, demonstrating saccular alveolar spaces (dark arrow) and under-alveolated acinar ducts (white arrow). (b) Z-stacks of images from confocal microscopy. A commercial PET membrane (DOW Corning) (10 µm thick) with 0.4 µm pore size, seeded with hAELVi cells, phalloidin actin staining (white). (c) Upper panel, Exploded and assembled computer-aided drawing (CAD) views of a pulmonary tree-on-a-chip device. Bottom panel, view of a complete pulmonary tree-on-a-chip device.

TROPOELASTIN COATED PLLA-PLGA SCAFFOLDS PROMOTE VASCULAR NETWORK FORMATION

Shira Landau, Department of Biomedical Engineering, Technion-Israel Institute of Technology, Israel
Email: Shiralan@tx.technion.ac.il
Ariel A. Szklanny, Department of Biomedical Engineering, Technion-Israel Institute of Technology, Israel
Giselle C. Yeo, Charles Perkins Centre, University of Sydney, NSW 2006, Australia
Yulia Shandalov, Department of Biomedical Engineering, Technion-Israel Institute of Technology, Israel
Anthony S. Weiss, Charles Perkins Centre, University of Sydney, NSW 2006, Australia
Shulamit Levenberg, Department of Biomedical Engineering, Technion-Israel Institute of Technology, Israel

Key Words: Tropoelastin, Vascularization, Wound repair

The robust repair of large wounds and tissue defects relies on blood flow. This vascularization is the major challenge faced by tissue engineering on the path to forming viable thick, implantable tissue constructs. Without the presence of this vascular network, oxygen and nutrients cannot reach the cells located far from host blood vessels. To create viable constructs, tissue engineering takes advantage of the mechanical properties of synthetic materials, while combining them with extracellular matrix (ECM) proteins to create a natural environment for the tissue-specific cells. Tropoelastin, the precursor of the elastin, is the ECM protein responsible for elasticity in all elastic tissues in the body, including robust blood vessels. We seeded human adipose microvascular endothelial cells (HAMECs) combined with mesenchymal stem cells (MSCs) on poly(L-lactic acid)/poly(lactide-co-glycolide) (PLLA/PLGA) scaffolds treated with tropoelastin, and examined the morphology, expansion and maturity of the newly formed vessels. Our in vitro results demonstrate that the treated scaffolds show a more expanded, complex and developed vascularization, in comparison to the untreated control group. To further explore the benefits of tropoelastin-treated scaffolds, we implanted pre-vascularized constructs within the mouse abdominal wall muscle by replacing a piece of the muscle with the engineered constructs. Within 12 days after implantation, we saw enhanced perfusion by host blood vessels. These results point to the great potential of these combined materials in promoting the vascularization of implanted tissue engineered constructs.

ENGINEERED NANOTHERAPEUTICS FOR PULMONARY AEROSOL DELIVERY

Catherine Fromen, University of Delaware, Chemical Engineering
cfromen@udel.edu
Bader Jarai, Zachary Stillman, Jeffery Noble, Anthony Zimmerman, University of Michigan

Key Words: pulmonary delivery, nanomedicine, immune engineering

Despite centuries of use and widespread application, aerosol delivery of therapeutics remains limited to a small subset of diseases and active pharmaceutical ingredients, mainly restricted to small molecule delivery for asthma management. Respiratory diseases which would benefit from direct and localized treatment span a much larger landscape; chronic obstructive pulmonary disease (COPD), lower respiratory infections, and lung cancers alone globally contribute 7.8 million annual deaths, with a reported 117 million pulmonary cases (~37% of population, 2012) and over $88 billion in health care costs in the US[1, 2]. To expand the application of aerosol delivery, novel approaches are needed. To address this need, we have explored various applications of nanoparticle immune engineering for respiratory therapeutics[3]. Incorrect immune responses lie at the heart of most respiratory diseases and advances in these therapeutic areas requires consideration of the unique environment. Notably, the lung has an abundance of antigen presenting cells (APCs), such as macrophages and dendritic cells (DC), which phagocytose foreign materials at the air-lung interface. There are a number of lung-specific APC populations[4, 5]. Some subsets are well understood, however, other specialized subsets have only recently been identified due to historic challenges in differentiating these populations[6, 7]. Thus, there are many remaining questions as to the division of labor between these cells, their significance in different disease conditions, and their interactions with other adjacent cell populations at the mucosal interface[8]. Advancing this understanding is critical to develop new therapeutics; APCs are poised as the gatekeepers to lung regulation and lung DC-subset specifically are likely cellular targets for therapeutic intervention[9].
In order to better understand how these lung innate immune cells respond to inhaled particle therapeutics, we have developed sets of engineered particles with defined physical properties that originate at the molecular level. We have developed a series of metal organic framework (MOF) nanoparticle carriers with independently tunable particle size and internal porosity, enabling systematic investigation of the effect of particle pore structure on cellular interactions. These UIO-66 MOF derivatives have not only been optimized as pulmonary aerosol carriers but provide critical insight on the role of internal particle porosity following cellular internalization. To further modulate the lung immune environment and evaluate the role of ligand surface density on immune-modulation, we simultaneously developed a series of degradable polymeric nanoparticle carriers with controlled surface densities of two Toll-like receptor (TLR) ligands, lipopolysaccharide (LPS), corresponding to TLR-4, and CpG oligodeoxynucleotide, corresponding to TLR-9[10]. Our *in vitro* results with murine bone marrow derived macrophages and *in vivo* studies following a direct instillation to murine airways both support a trade-off between particle dosage and optimal surface density; proinflammatory cytokine production was driven by the distribution of the adjuvant dose to a maximal number of innate cells, whereas the upregulation of costimulatory molecules on individual cells required an optimal density of TLR ligand on the particle surface. Taken together, results from these two sets of particle types demonstrate that both particle porosity and ligand surface density are critical parameters for tight control of immune stimulation and association with lung APCs and provide a foundation to build pathogen mimicking particle (PMP) vaccines and immunostimulatory therapeutics.

References:
1. WHO: World Health Organization 2012.
2. NIH: National Heart, Lung, and Blood Institute 2012.
3. Moon, J. J.; Huang, B.; Irvine, D. J., Advanced materials (Deerfield Beach, Fla.) 2012, 24 (28), 3724-46.
4. Guilliams, M.; Lambrecht, B. N.; Hammad, H., Mucosal Immunol 2013, 6 (3), 464-73.
5. Kopf, M.; Schneider, C.; Nobs, S. P., Nat Immunol 2015, 16 (1), 36-44.
6. Blank, F.; Stumbles, P. A.; Seydoux, E.; Holt, P. G.; Fink, A.; Rothen-Rutishauser, B.; Strickland, D. H.; von Garnier, C., Am J Respir Cell Mol Biol 2013, 49 (1), 67-77.
7. Fytianos, K.; Drasler, B.; Blank, F.; von Garnier, C.; Seydoux, E.; Rodriguez-Lorenzo, L.; Petri-Fink, A.; Rothen-Rutishauser, B., Nanomedicine (Lond) 2016, 11 (18), 2457-2469.
8. Hasenberg, M.; Stegemann-Koniszewski, S.; Gunzer, M., Immunol Rev 2013, 25 (1), 189-214.
9. Zhao, L.; Seth, A.; Wibowo, N.; Zhao, C. X.; Mitter, N.; Yu, C.; Middelberg, A. P., Vaccine 2014, 32 (3), 327-37.
10. Noble, J.; Zimmerman, A.; Fromen, C. A., ACS Biomater Sci Eng 2017, 3 (4), 560-571.

MODULAR CONTROL OF INNATE IMMUNE SIGNALING USING SELF-ASSEMBLY OF IMMUNE SIGNALS

Christopher M. Jewell, Fischell Department of Bioengineering, University of Maryland – College Park
cmjewell@umd.edu

Key Words: adjuvant, vaccine, immunology, nanotechnology, cancer

Vaccines play an increasingly important role in preventing and treating diseases ranging from infectious pathogens to cancer because these technologies harness the specificity of the immune system to clear disease without targeting the body's own cells. To realize these goals, new understanding of adjuvants – molecules added to vaccines to enhance function – is needed to support design of next-generation vaccines that elicit responses tailored for specific diseases. We recently reported a simple nanotechnology platform based on self-assembly of peptide antigen and a molecular toll-like receptor agonist (TLRa) to create modular vaccine designs (*ACS Nano* 2016, *ACS Nano* 2015). These structures – termed immune polyelectrolyte multilayers (iPEMs) – juxtapose antigen and TLRa at high densities, and offer 100% cargo loading since no carrier component is needed. This modularity also creates the possibility of rationally designing iPEMs that trigger multiple immune pathways with distinct control over the relative activation levels. In cancer, for example, activating multiple innate pathways has been linked to improved patient outcomes in human clinical trials. To exploit iPEMs in this manner, we designed iPEM architectures incorporating a conserved human cancer antigen (Trp2), and a range of molecularly-defined TLRa that spanned different TLRa classes and species (i.e., mouse and human): agonists for TLR3, TLR9, and TLR13. iPEMs were assembled from Trp2 and one, two, or three TLRas, or alternatively, using two different TLRas at varying compositions. To form carrier free capsules using these design schemes, Trp2 was appended with cationic amino acids, then adsorbed onto a sacrificial colloidal template, with alternating adsorption steps employing the specified TLRas (anionic). Centrifugation and wash steps were performed after each adsorption, then the template was dissolved using a chelator (EDTA) to form carrier-free capsules formed entirely from tumor peptide and each TLRa composition. All components were labeled to facilitate measurement of composition by fluorimetry and confocal microscopy. Using this approach, we discovered iPEMs could be assembled from any combination of Trp2 and the TLRas (**Fig. 1A**). Quantification revealed further confirmed iPEM capsules consisting of the corresponding peptide antigen and TLRa ligands (**Fig. 1B**). iPEMs incubated with primary dendritic cells isolated from BL6 mice revealed a high degree of colocalization of each iPEM component within cells. For example, iPEMs consisting of Trp2 and all three TLRas revealed that 90% of cells positive for at least one iPEM component were positive for all four components (**Fig. 1C**). Compared with equivalent free mixtures, iPEMs drove synergistic activation of DCs measured using flow cytometry for common surface activation makers (e.g., CD80, CD40, CD86) (**Fig. 1D**). Importantly, iPEMs also allowed modular control of TLR signaling, revealed using iPEMs built from Trp2 while varying the input ratio of TLR3a:TLR9a to control the final composition. iPEMs with a high TLR3a:TLR9a ratio triggered a high level of TLR3 signaling (**Fig. 1E**). As the ratio decreased, TLR3a signaling decreased, while TLR9a signaling increased. This rational control could contribute to more effective vaccines that use molecular adjuvant assembly to direct specific combinations and levels of multiple innate signaling pathways.

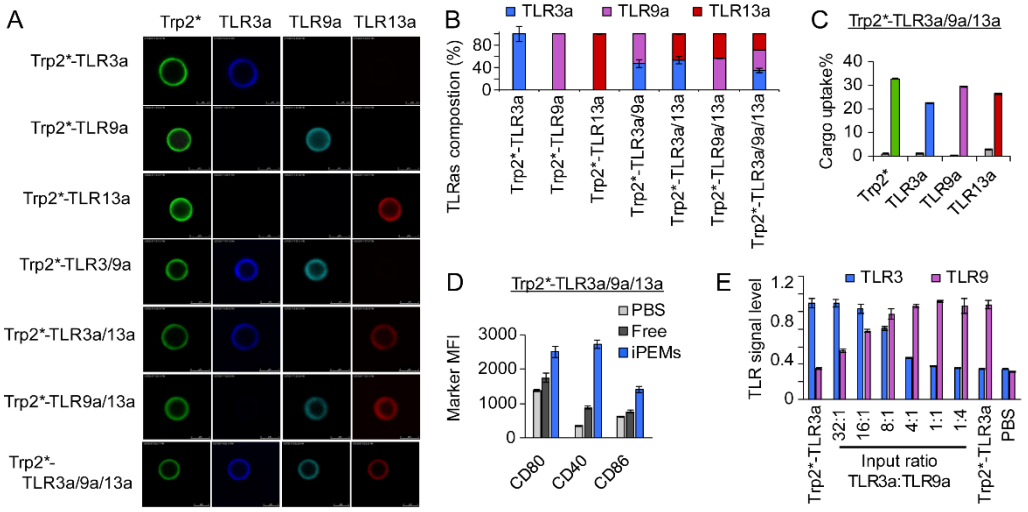


Figure 3 – A) Modular assembly of Trp2 and combinations of TLRas in iPEMs. B) Quantification of TLRa loading. C) iPEMs deliver multiple signals to DCs, and D) drive synergistic responses relative to equivalent free signals. E) iPEMs can be prepared with distinct TLRa compositions, leading to tunable activation of each TLR signaling pathway.

A MICROFLUIDIC-COMPUTATIONAL PLATFORM FOR ANALYZING VASCULAR AND EXTRAVASCULAR MASS TRANSPORT

Hilaria Mollica, Istituto Italiano di Tecnologia,
hilaria.mollica@iit.it
Pietro Lenarda, Istituto Italiano di Tecnologia,
Roberto Palomba, Istituto Italiano di Tecnologia,
Rui Pereira, Istituto Italiano di Tecnologia
Paolo Decuzzi, Istituto Italiano di Tecnologia

Key Words: microfluidic chips; computational modeling; Drug Delivery; Membrane

Considerable advances have been made in microfluidic devices and their applications since the development of soft lithographic techniques [1]. We developed a PDMS based double channel chip consisting of two microfluidic channels that mimic the vascular and extravascular compartments. The two channels are designed to be confined by sidewalls and connected by a membrane composed by arrays of pillars constituting a permeable vascular wall [2]. The inner surface of the vascular channel is uniformly coated with Human Umbilical Vein Endothelial Cells (HUVEC) resulting in well-controlled 3D model of blood vessel with endothelial barrier functions. In **Figure.1A** and **B**, the photolithographic, etching, and replica molding steps needed for realizing double-channel chips are presented together with an image (right) of the vascular channel after cell seeding and self-organization in a tubular shape. The extravascular compartment can be integrated with tumor cells of different type, potentially organized in a 3D fashion inside an extracellular matrix or with extracellular matrix components. The integration of the two compartments allow us to study the transport and permeation of therapeutic molecules, nanomedicines and cells through the endothelial barrier and the efficacy of the administered treatment. Other applications such as modeling of metastatic cell and leucocytes adhesion and migration across the endothelial barrier allow us to characterize cell extravasation from the vascular bed. The vascular transport and subsequent adhesion dynamics of nano-constructs and cells to the vascular channel are also predicted using a 3D computational framework based on coupling Lattice Boltzmann (LB) and Immersed Boundary (IB) methods. The fluid solver for the incompressible Navier-Stokes equations is based on the three dimensional D3Q19 Lattice-Boltzmann Method. The dynamics of deformable nano-constructs and cells is simulated through a neo-Hookean membrane constitutive model coupled iteratively with the fluid (**Figure.1C**). The combination of microfluidic chips and computational modeling provides a formidable tool for boosting our understanding on disease development and drug delivery.

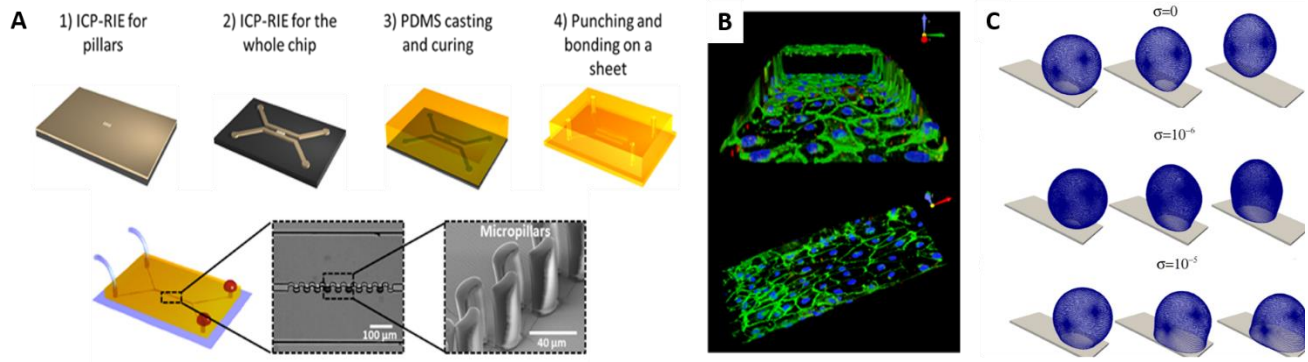


Figure 1. Double-channel microfluidic chips. A. Fabrication steps and SEM images. B. Confocal images of a confluent HUVEC monolayer cultured in the vascular compartment, where nuclei are stained in blue and VE-cadherin receptors are stained in green. C. Adhesive interactions at the wall and deformation of a circulating cell.

ESSENTIAL REFERENCES
1.Bhatia, S. N.; Ingber, D. E. Microfluidic organs-on-chips. *Nat. Biotechnol.* 2014, 32, 760-772.
2.Manneschi, C.; Pereira, R. C.; Marinaro, G.; Bosca, A.; Francardi, M.; Decuzzi, P. A microfluidic platform with permeable walls for the analysis of vascular and extravascular mass transport. *Microfluid Nanofluid*, 2016, 20.
3.Coclite, A., Mollica H., Ranaldo S., Pascazio G., de Tullio M.D., Decuzzi P. Predicting different adhesive regimens of circulating particles at blood capillary walls. *Microfluidics and Nanofluidics*, 2017. **21**(11): p. 168.

TARGETED DRUG DELIVERY IN ARTERIAL STENOSIS - ROLE OF HEMODYNAMICS

Moran Levi, Faculty of Biomedical Engineering, Technion, Israel
smoranle@campus.technion.ac.il
Mark Epstein, Faculty of Biomedical Engineering, Technion, Israel
Netanel Korin, Faculty of Biomedical Engineering, Technion, Israel

Key Words: Hemodynamics, Stenosis, Drug Delivery

Hemodynamics play a central role in cardiovascular targeted drug delivery systems. Sites of abnormal vascular narrowing (stenosis) exhibit unique flow features including an abnormally elevated level of shear stress as well re-circulating flows downstream of the narrowing. Here we study the deposition of particulate drug carries in models of arterial stenosis under defined hemodynamic conditions.

First, fluorescently tagged Poly (Lactic-co-Glycolic Acid) (PLGA) nano-particles functionalized with collagen targeting motifs have been fabricated. Then experiments in a microfluidic channels coated with collagen were performed to characterize the adhesion properties of the examined particles as a function of shear. Next, perfusion experiments on collagen coated millimeter sized vascular models of stenotic coronary arteries have been performed using a custom-built perfusion system capable of emulating pulsatile physiological flow. The particle deposition was monitored using time-lapse fluorescence microscopy at defined locations within the model. Our results show that particle size and coating density affect the deposition pattern within the stenosis and that there is correlation between the microfluidic results under defined shear stress and the deposition in the stenosis models. Altogether our results illustrate the key role of hemodynamics in designing cardiovascular nano-medicines.
.

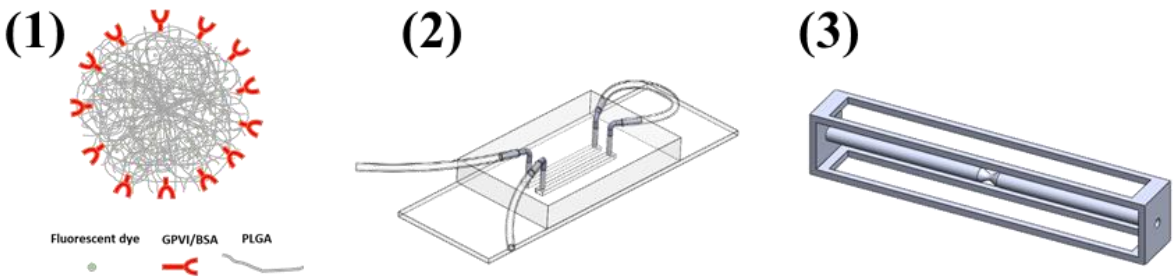


Figure 4 – Scheme representing the different stages of the study. (1) PLGA particle conjugated with collagen binding motifs (2) A CAD (computer aided design) model of microfluidic channels used to test particle adhesion to defined surfaces under constant flow (3) A stenosis CAD model showing the geometry of the model to study adhesion in a stenosis site.

STUDY CELLULAR RESPONSES AT THE MICROSCALE BY CREATING HETEROGENEITY IN CULTURED CELLS USING A MICROFLUIDIC PROBE

Nadia E. Casimiro[1,2], Gema V. Gonzalez[1,3], Aditya Kashyap[1,3], Lena V. von Voithenberg[1], Govind V. Kaigala[1]
[1]IBM Research – Zurich, CH; [2]University of Basel, CH; [3]ETH Zurich, CH

Key Words: *cell lysis, gene expression, microfluidics, heterogeneity in cancer, drug resistance*

We introduce a new approach to study cellular responses in different cell subpopulations while not disrupting the microenvironments. We believe this might become a useful tool to investigate resistance-related cellular responses in cancer cells.

Drug resistance of cancers is currently one of the defining issues in providing effective treatment to patients. The heterogeneity in cell responses to a therapeutic agent is challenging to deconvolve not only owing to neo-antigens that arise in a tumor, but also due to complexity of interconnected molecular events that occur in a cell, making it difficult to decipher by which mechanism(s) they survived the treatment. There is therefore a need to develop new *in-vitro* models taking into account cancer heterogeneity and sub-population cross talk. Spatial localization of stimuli/insult and study of spatial cues provide one such avenue that may allow to study the effect of them on cancer cell microenvironments and provide meaningful information to help understand cellular behavior.In the last years, the microfluidics community has introduced different *in-vitro* models with organs-on-a-chip research and new analytical methods for such applications. However, the transfer of cancer *in vitro* models into microfluidics has faced some limitations to reproduce the complex microenvironment and subpopulations of cells found in a cancer niche. Interestingly, a new class of microfluidic devices called 'open space microfluidics' - in contrast to traditional microfluidic devices - enables probing cells without disrupting the biological surface, thereby preserving the natural microenvironment of the cells/tissue.The microfluidic probe (MFP) is one implementation of open space microfluidics that consists of a microfabricated probe head that locally confines and shapes nanoliter volumes of multiple reagents on a biological surface. Liquid localization is achieved by simultaneous injection and aspiration of the reagent from proximate apertures while the apex of the MFP maintains a fixed distance from an immersed surface (hydrodynamic flow confinement - HFC). These confined reagents can be stimulants, insults or biochemicals in molecular biology assays.We are developing assays to create heterogeneity on cultured surfaces by means of local treatment of cells. We are combining spatial lysis sequentially within the same assay to study gene expression signatures of the treated and untreated populations on the same biological substrate. While studying the uptake of small molecules such as Calcein-AM, we observed faster uptake (3.5×) of Calcein in MCF-7 cells when compared to bulk assays, which we will use to our advantage for shortening treatment times to create heterogeneity. In addition, we have formulated different lysis solutions locally confined with the MFP for RNA isolation. Using a Triton-X detergent and proteinase K lysing solution, we are able to obtain RNA containing lysates in less than 1 min at room temperature. We are translating the Calcein-AM assay for EGF loading to obtain gene expression signatures of proliferation indices such as *c-fos*

Spatial localization on biological substrates is an attractive approach to apply in cancer research. The advantages to create heterogeneity and supply more the one chemical at a time in shorter times make it suitable to think that we can perform synergistic and antagonistic treatments while studying their toxic effects on cancer cells.

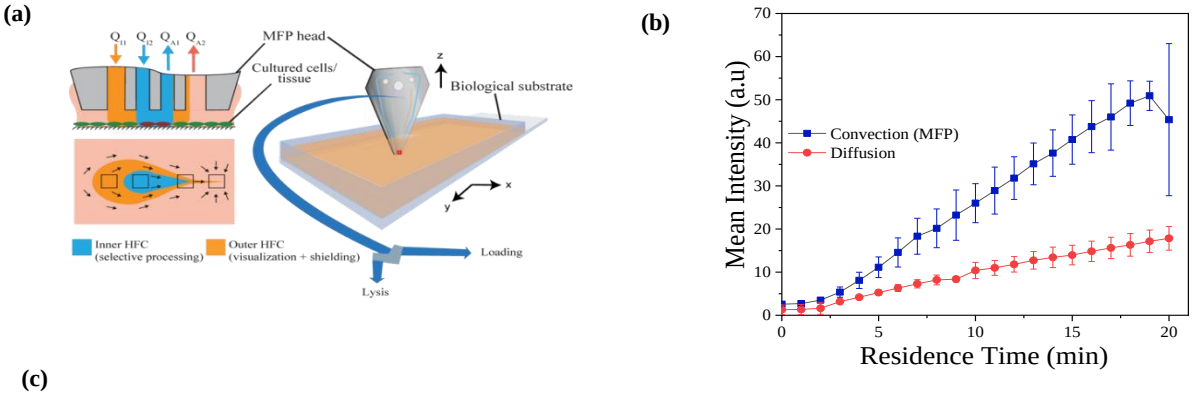


Figure 1 (a) Working principle of the MFP (b) Comparison of Calcein-AM loading with MFP versus bulk assays in MCF-7 cells (c) Spatial lyse for local RNA isolation from of cells tracked with CMFDA Dyes.

SYNTHETIC CELLS SYNTHESIZE THERAPEUTIC PROTEINS INSIDE TUMORS

Nitzan Krinsky, Laboratory for Targeted Drug Delivery and Personalized Medicine Technologies, Department of Chemical Engineering, Technion, Haifa, Israel.
nitzank@technion.ac.il
Maya Kaduri, Laboratory for Targeted Drug Delivery and Personalized Medicine Technologies, Department of Chemical Engineering, Technion, Haifa, Israel.
Assaf Zinger, Laboratory for Targeted Drug Delivery and Personalized Medicine Technologies, Department of Chemical Engineering, Technion, Haifa, Israel.
Janna Shainsky-Roitman, Laboratory for Targeted Drug Delivery and Personalized Medicine Technologies, Department of Chemical Engineering, Technion, Haifa, Israel.
Mor Goldfeder, Laboratory for Targeted Drug Delivery and Personalized Medicine Technologies, Department of Chemical Engineering, Technion, Haifa, Israel.
Itai Benhar, Department of Molecular Microbiology and Biotechnology, The Georg S. Wise Faculty of Life Sciences, Tel-Aviv University, Tel-Aviv, Israel.
Dov Hershkovitz, Department of Pathology, Tel-Aviv Sourasky Medical Center, Tel Aviv, Israel
Avi Schroeder, Laboratory for Targeted Drug Delivery and Personalized Medicine Technologies, Department of Chemical Engineering, Technion, Haifa, Israel.

Key Words: nanotechnology, personalized medicine, protein drugs, protocells, synthetic cells.

The existing dogma is that protein medicines need to be produced in large factories, and then injected to the patient. We propose that miniature artificial inert factories can be injected to the patient, to produce a protein of interest directly in the diseased tissue. We engineered artificial cell-like particles with an autonomous capacity to synthesize protein drugs after receiving an external signal. The protein is tuned to the patient's needs based on a predetermined DNA code we incorporate inside the particles. This approach increases treatment efficiency and reduces adverse effects to healthy tissues.
We developed a new T7-S30 based cell-free protein synthesis system, which contains all the transcription and translation machines and molecules required for protein production (Krinsky *et al*., *PloS one* 2016). This system was used to prepare liposomes that act as artificial cells, capable of producing proteins autonomously in response to a physical trigger. Functional enzymes (luciferase and tyrosinase) and fluorescent proteins (GFP) were successfully produced using the new cell-free protein synthesis system and inside the particles both *in vitro* and *in vivo*. In addition, we demonstrated the therapeutic capabilities of the protein producing particles by producing *Pseudomonas* exotoxin A, an extremely potent protein, for treating cancer. Applying the particles on 4T1 cells (a triple-negative breast cancer cell-line) *in vitro* or injecting them into a 4T1-induced tumor *in vivo*, resulted in high cytotoxicity due to the effective production of the therapeutic protein inside the vesicles (Krinsky *et al*. *Advanced Healthcare Materials,* 2017).
Synthetic cells serve as autonomous, trigger-able, artificial particles that produces a variety of proteins. This platform has promise to address a wide range of fundamental questions associated with protein synthesis in nature, as well as applicative protein delivery needs.

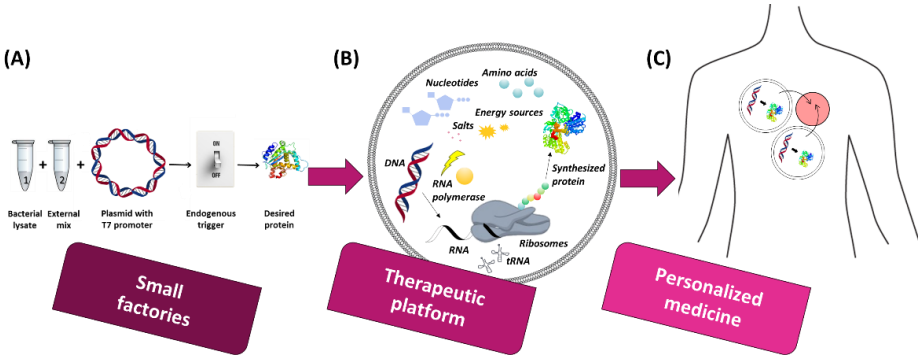


Figure 1 - the therapeutic platform. Onsite synthesis of protein medicines inside the body is achieved by encapsulation of all the transcription and translation factors required for protein production inside a liposome. (A) Cell-free protein synthesis (CFPS) work flow. (B) Protein producing particle. (C) Personalized medicine; triggered protein production inside the patient's body at the diseased tissue.

CONTROLLED EGFR LIGAND DISPLAY FOR TUNABLE TARGETED INTRACELLULAR DELIVERY OF CANCER SUICIDE ENZYMES

Rachel Lieser, University of Delaware
rmlieser@udel.edu
Wilfred Chen, University of Delaware
Millicent Sullivan, University of Delaware

Key Words: Intracellular delivery; Protein engineering; Cancer

Advances in molecular engineering have led to customizable proteins for a multitude of clinical applications, leading to the rapid growth of the protein pharmaceutical market. Proteins can be advantageous compared to other treatment methods because of their functional complexities and high specificity that cannot be mimicked by small molecule drugs. Although there has been great interest and investment in advanced protein therapeutics, a majority of marketed proteins continue to have extracellular targets, despite the therapeutic relevance for intracellular protein therapies. Engineering efforts to improve cytosolic protein delivery often rely on modifying proteins through direct conjugation of polymers and peptides using reactive residues on amino acids. While this method has shown some success, the inability to modify a specific site within a protein can significantly hinder pharmacological action. Additionally, such approaches do not offer control over design variables that can be important determinants of targeting efficacy, such as ligand clustering.

Previous work has demonstrated the ability to site-specifically insert biorthogonal reactive residues into proteins through unnatural amino acid incorporation, enabling direct protein conjugation with simple ‘click’ chemistries. Such an approach could be applied to protein therapeutics to explore the effect arrangement of delivery molecules has on protein bioactivity and intracellular delivery capabilities. In our work, we have demonstrated application of this approach for conjugation of epidermal growth factor receptor (EGFR) targeting peptides in fluorescent proteins. By varying EGFR peptide arrangements we have demonstrated the ability to tune cellular internalization in inflammatory breast cancer (IBC) cells. Furthermore, this system has been adapted for delivery of a cancer suicide enzyme to enable IBC-targeted cell death through prodrug activation. Through this approach, we have identified the importance of ligand display for targeted protein delivery and applied these finding to enhance enzyme delivery to IBC cells. Future efforts will refine the efficacy of this method though incorporation of endosomal escaping peptides and hydrophilic polymers to address additional challenges associated with *in vivo* intracellular protein delivery.

DEVELOPMENT OF A COLLAGEN-BASED SCAFFOLD FOR SEQUENTIAL DELIVERY OF ANTIMICROBIAL AGENTS AND PDGF GENES TO CHRONIC WOUNDS

Raj Kumar Thapa, Department of Chemical and Biomolecular Engineering, University of Delaware, USA
thaparaj@udel.edu
Kristi L. Kiick, Department of Material Science and Engineering, University of Delaware, USA
Millicent O. Sullivan, Department of Chemical and Biomolecular Engineering, University of Delaware, USA

Key Words: Antimicrobial agent, chonic wound, collagen, gene, growth factor

Chronic wounds are a global health burden affecting more than 5 million people in the United States alone. The complex wound microenvironment causes variable therapeutic outcomes following treatment with commercially available products. Wound infection is one of the major barriers in healing of wounds and localized delivery of antimicrobials is necessary for treatment. Furthermore, growth factors play a vital role in orchestrating the wound healing process through enhancement of cell proliferation, migration, and extracellular matrix remodeling. Accordingly, we have developed a collagen-based scaffold modified with combination of vancomycin-loaded liposomes and platelet derived growth factor (PDGF)-loaded DNA polyplexes. Both the liposomes and polyplexes were anchored to collagen using collagen mimetic peptides (CMPs). Our aim was to use CMP tethering to control the sequential release of vancomycin and PDGF polyplexes to immediately suppress infection and subsequently transfect wound bed fibroblasts with PDGF to assist the wound healing process. Vancomycin-loaded liposomes were prepared using dipalmitoylphosphatidylcholine (DPPC), cholesterol, and 1,2-distearoyl-sn-glycero-3-phosphoethanolamine-N-[amino(polyethylene glycol)-2000] (DSPE-PEG). The liposomes were 160.7±2.1 nm in diameter based on dynamic light scattering (DLS) analyses, and the loading capacity of vancomycin was 51.5±0.7% in the liposomes. PDGF polyplexes (115.2±1.2 nm in diameter) were prepared by self-assembly of polyethyleneimine and PDGF plasmid DNA (N/P = 8) in 20 mM HEPES buffer (pH = 6.0), and successful PDGF gene loading was confirmed by agarose gel electrophoresis. Co-gels were prepared with collagen (4 mg/mL), fibrinogen (1.25 mg/mL), and thrombin (0.156 IU/mL) combinations that could successfully encapsulate both the vancomycin-loaded liposomes and PDGF polyplexes. Drug release studies confirmed that ~80% of the vancomycin was released during the 48 h study period, whereas PDGF polyplexes were retained longer (> 5 days) in the gel because their release requires collagen degradation mediated by matrix metalloproteinases present in the wound bed. The ability of the PDGF polyplexes to transfect fibroblasts was confirmed by *in vitro* cell transfection studies using green fluorescent protein (GFP) as a model gene. Furthermore, polyplex-mediated PDGF transfection was evaluated in fibroblasts cultured in an *in vitro* culture wound model, which showed that PDGF transfection enhanced migration rates of fibroblasts by ~2.4 fold as compared to controls in which culture wounds were allowed to heal in the absence of polyplexes. These results showcase the capacity for sequential delivery of vancomycin and PDGF gene *in vitro*, using collagen-based scaffolds, for potential applications in *in vivo* chronic wound treatments.

SYNTHESIS OF ZWITTERIONIC-FUNCTIONALIZED CONJUGATED NANOPARTICLES FOR TARGETED DRUG DELIVERY APPLICATIONS

Renato Auriemma, Politecnico di Milano, Milan, Italy
Renato.auriemma@polimi.it
Umberto Capasso Palmiero, Politecnico di Milano, Milan, Italy
Davide Moscatelli, Politecnico di Milano, Milan, Italy
Marco Peviani, Dana-Farber/Boston Children's Cancer and Blood Disorders Center, Boston,MA, USA

Key Words: ligands, Zwitterionic nanoparticles, RAFT polymerization, nanomedicine

Polymeric Nanoparticles (NPs) represent a promising pharmacological tool, since their structure can be modified to obtain: i) encapsulation and controlled release of a wide range of active compounds, ranging from small molecules to siRNA or oligonucleotides; ii) selective cell targeting, thus allowing precise drug delivery to the desired site of action. A powerful strategy to achieve selectivity of uptake in specific cell types is to conjugate the nanoparticles to a ligand specific for receptors expressed by the target cell type. This offers the advantage of a potentially improved drug efficacy with limited side effects and toxicity.

Polymeric nanoparticles in a range of 20–100 nm have a high potential for in vivo applications, due to their ability to circulate in the blood for a long period of time. In fact, this size range allows to avoid renal and lymphatic clearance, to prevent opsonization and at the same time improves the internalization by cells.

In this work we address the synthesis by reversible addition-fragmentation chain transfer (RAFT) of biodegradable, zwitterionic-based nanoparticles. This Zwitterionic nanoparticles act as super non-fouling surfaces that prevent protein adsorption from complex biological media. The nanoparticles were functionalized with different numbers of selective ligands through click chemistry; different dimensions were synthetized changing the length of the hydrophobic part. In vitro studies were performed to evaluate the uptake of functionalized nanoparticles.

TRUE-SCALE BIOMIMETIC MULTI-GENERATION AIRWAY PLATFORMS OF THE HUMAN BRONCHIAL EPITHELIUM FOR *IN VITRO* CYTOTOXICITY SCREENING

Shani Elias-Kirma, Department of Biomedical Engineering, Technion, Haifa, Israel.
shaniki@campus.technion.ac.il
Arbel Artzy-Schnirman, Department of Biomedical Engineering, Technion, Haifa, Israel.
Rami Fishler, Department of Biomedical Engineering, Technion, Haifa, Israel.
Netanel Korin, Department of Biomedical Engineering, Technion, Haifa, Israel.
Josué Sznitman, Department of Biomedical Engineering, Technion, Haifa, Israel.

Key Words: organ-on-chip, physiological flow, *in vitro*, air-liquid interface.

Lung exposure to inhaled particulate matter may injure the epithelial tissue and lead to a loss of function in affected regions via inflammation for example. Screening for the critical contaminate concentrations may provide essential information towards damage assessment and epithelial healing. To date, most approaches have typically relied on traditional *in vitro* well plate assays or alternatively *in vivo* animal experiments. Yet, such methods manifest some outstanding disadvantages such as the inability to capture physiological flow and aerosol deposition characteristics as well as significant differences in anatomy, immune system and inflammatory responses compared to humans. The advent of organ-on-chip platforms has shown promising results to reconcile many such drawbacks. In an attempt to provide an attractive *in vitro* gateway to monitor airway health, we discuss here a novel biomimetic platform which emulates the bronchial epithelium of a human upper airway, allowing to study organ-level characteristics in a homeostatic cellular microenvironment. This device reconstitutes a multi-generation pulmonary epithelial airway environment, capturing realistic respiratory transport phenomena and critical cellular barrier functions at an air-liquid interface (ALI), in analogy to the bronchial lumen. As a proof of concept, we demonstrate its feasibility for *in vitro* based assays by exposing the device to cytotoxic aerosolized particles under respiratory flow conditions. Subsequently, we investigate the cytotoxic effects of these particles including cellular viability, cytokine and mucus secretion as a function of local particle deposition patterns. Ultimately, our bronchial airway models are intended to provide off-the-shelf *in vitro* kits geared for the end-user interested in a wide range of broader biological assays that may be attractive for cytotoxicity and drug screening.

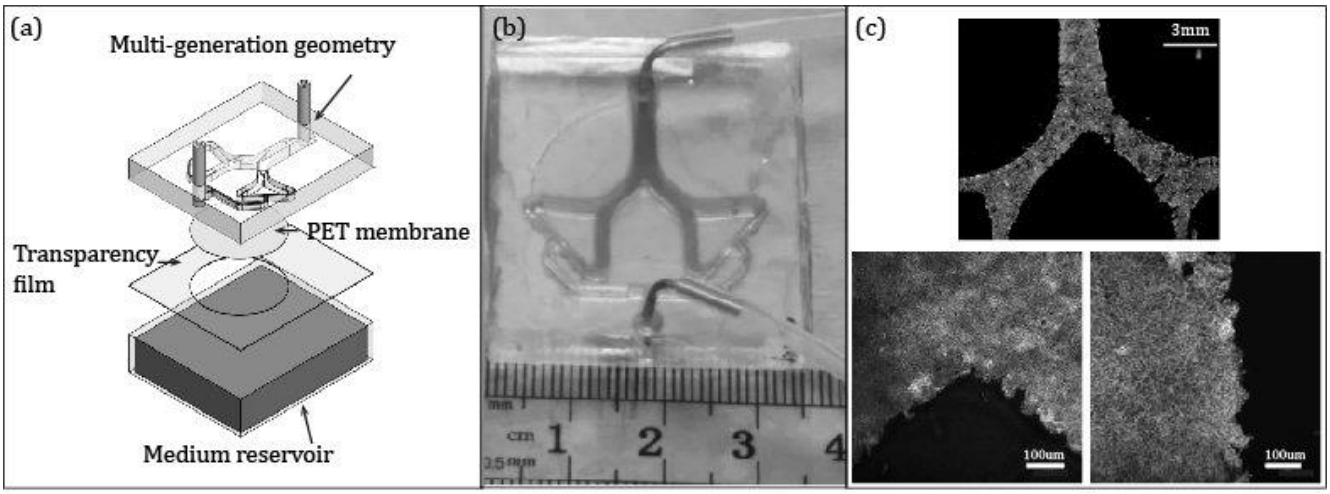


Figure 1: (a) Computer-aided drawing (CAD) of our model depicting a multi-generation airway tree, a PET membrane bonded with a transparency film that sits on a medium reservoir. (b) View of the whole device. (c) Calu-3 cells cultured at an ALI within the device: view of the entire tree (upper), the first bifurcation (bottom left) and the third branch (bottom right) of the structure (cells stained green for F-actin and blue for cell nuclei).

POLYESTER-BASED EXCIPIENTS TO FORMULATE LIPOPHILIC DRUGS INTO NANOPARTICLES DIRECTLY AT THE BED OF THE PATIENT

Umberto Capasso Palmiero, Politecnico di Milano, Milan, Italy
umberto.capasso@polimi.it
Massimo Morbidelli, ETH, Zurich, Switzerland
Maurizio D'Incalci, IRCCS Mario Negri, Milan, Italy
Davide Moscatelli, Politecnico di Milano, Milan, Italy

Key Words: nanoparticles, self-assembling copolymers, Trabectedin, Paclitaxel,

In recent decades there has been an increased interest in polymeric nanoparticles as drug delivery systems thanks to their several advantages, such as continuous maintenance of drug levels in a therapeutically desirable range, and reduction of harmful side effects. These nano-colloids are generally made up of polyesters as long as they are able to easily degrade into the body. However, NP production is often a process that requires complex microfluidic devices. In addition, expensive purification steps are necessary to eliminate the unloaded drug and the high amount of organic solvent used in the NP production step. In the end, a lyophilization step is general adopted to assure a good shelf-life of the final product. All the above-mentioned steps hamper the cost-effective use of a re-formulation of the same therapeutic agent and, in turn, reduce the availability of these treatments among the patient population. For this reason, in this work, a novel NP production protocol that consists only in the use of a syringe and a needle without the need of subsequent purification and freeze-drying steps has been developed. This has been possible by the optimization of the hydrophilic/lipophilic balance of block-copolymers that are able to directly self-assemble in water. The additional degree of freedom necessary for this optimization was introduced via the synthesis of these materials thorough the combination of the reversible addition-fragmentation chain transfer (RAFT) polymerization and the ring opening polymerization (ROP). The NPs has been used to formulate Trabectedin (ET-743), a widely adopted anticancer therapeutic known for its local adverse effect. The pharmacokinetic behavior, antitumor activity and toxicity of this novel NP-based formulation has been compared to the commercially available formulation Yondelis®. NPs have shown the ability to retain the drug into circulation for a longer time in the blood stream compared to the free ET-743 allowing to considerably reduce the local toxic effects. In addition, the shift of the NP preparation step from a specialist to the final user allows to avoid all the purifications and post-processing steps necessary to assure a good shelf-life of the product. In this way, a ET-743 formulation less toxic than the commercially available Yondelis® can be produced at a competitive price taking also into account that this expensive drug is not lost in any of the NP production steps here adopted. In order to prove the versatility of this novel technology, Paclitaxel (PTX), an anticancer therapeutic that it usually formulated with a toxic surfactant (Chremophor EL), have been also formulated into this NPs. In this way, a novel PTX formulation can be produced at a lower cost compared to the ones already approved and present into the market. In particular, it has shown the same advantage in reduction of the toxicity given by the elimination of the Chremophor EL (e.g in Abraxane® and Genexol®).

ADHESION KINETICS OF FUNCTIONALIZED NANO-PARTICLES UNDER HIGH SHEAR CONDITIONS

Yathreb Assad, Faculty of Biomedical Engineering, Technion, Israel
yathreb@campus.technion.ac.il
Netanel Korin, Faculty of Biomedical Engineering, Technion, Israel

Key Words: Hemodynamics, Platelets, Functionalized Nano-Particles

Arterial thrombosis, the formation of blood clot within an artery, is a complex process that consists of multiple steps. These steps are orchestrated by three main factors: high shear flow, prothrombogenic surface (collagen), and blood components mainly von Willebrand factor (vWF) and platelets. One of the essential early events in thrombus initiation under high shear conditions are the interactions between vWF and platelet's receptor GPIbα. Here we study the adhesion kinetics of platelet's GPIbα receptor functionalized-nano particles on different coated-channels under defined hemodynamic conditions.

Polystyrene particles of 200nm were functionalized with Glycocalicin, the extracellular domain of platelet's GPIbα receptor, and bovine serum albumin (BSA). These functionalized NPs were perfused on vWF and collagen-coated channels, while their deposition were quantitatively evaluated by time-lapse fluorescence microscopy. Our results show that glycocalicin-coated particles exhibit shear-stress enhanced adhesion unlike BSA particlesand tht their adhesion is more selective vWF coated-channels compared with collagen coated-channels.
.

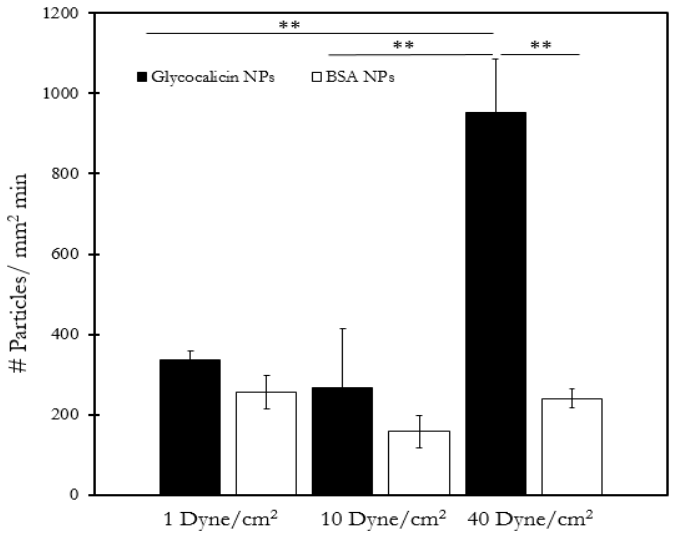


Figure 5 – Glycocalicin and BSA-Functionalized NPs adhesion rate on vWF -coated channels at different shear stresses.

TOLERANCE INDUCTION WITH QUANTUM DOTS DISPLAYING TUNABLE DENSITIES OF SELF-ANTIGEN

Krystina L. Hess, University of Maryland - College Park
klhess@umd.edu
Christopher M. Jewell, University of Maryland - College Park, University of Maryland Medical School, Marlene and Stewart Greenebaum Cancer Center, United States Department of Veterans Affairs

Key Words: autoimmunity, tolerance, quantum dots, immunotherapy, theranostics

During autoimmune diseases like type 1 diabetes or multiple sclerosis (MS), the immune system mistakenly recognizes and attacks healthy tissues in the body. In MS, myelin, which surrounds and protects the axons of neurons, is attacked by inflammatory cells leading to neurodegeneration. The current standard of care for MS patients is regular injection of immunosuppressive drugs that non-specifically suppress immune function, leaving patients immunocompromised and open to opportunistic infection. New investigations aim to address this problem with immunotherapy-based strategies that promote myelin-specific tolerance. Recent reports reveal that the development of inflammation or tolerance against certain molecules is influenced by the concentration and form of self-antigen presented to immune cells (i.e. free, particle). Strategies that allow tunable delivery of self-antigen are therefore of great interest to further probe these connections. Quantum dots (QDs) were chosen as the nanomaterial to investigate these questions because they can be conjugated with a large and controllable number of biomolecules. Additionally, their size facilitates rapid drainage through lymphatics to lymph nodes (LNs), where they accumulate and can be visualized by deep-tissue imaging due to their intrinsic fluorescence. QDs could be decorated with up to 130 myelin oligodendrocyte glycoprotein (MOG) peptides, a known self-antigen of MS (**Fig 1A**). In a mouse model of MS (EAE), we treated groups with two different doses of self-antigen conjugated to QDs. Treatment resulted in a significant reduction in paralysis that was dependent on MOG dose. At a time point just before EAE symptoms arise (day 9), it was discovered that the draining inguinal LN in MOG-QD treated mice had a significantly higher number of regulatory T cells (T_{REGS}) than those of untreated mice (**Fig 1B**). This response was also dose dependent, as mice receiving a higher dose of self-antigen on QDs (52:1) had more T_{REGS} than those injected with a lower dose (17:1). This cell population can induce tolerance to self-antigens by controlling inflammatory cells. We next tested if the level of tolerance induced was dependent on peptide dose alone or if ligand density had an effect. We treated groups with the same dose of MOG spread out over different amounts of QDs and found a trend of decreasing disease severity with decreasing ligand density (**Fig 1C**). This result inspired us to further investigate the trafficking of peptide decorated QDs and the effect that these nanomaterials have on the LNs they accumulate in. Initial studies indicate that we can track the biodistribution of MOG-QDs in naïve mice over time by flow cytometry. Strong QD signal is visible just one day post injection (*p.i.*), and peaks in the draining left inguinal LN on day 3 (**Fig 1D**). While this signal then wanes over the next 4 days, it sustains at a high level in the left axillary LN up to 1 week after injection. Interestingly, a much lower level of QD signal is seen in the right inguinal (non-draining) LN. We also quantified changes in cell populations over time following MOG-QD injection and discovered a spike in macrophages in the draining inguinal LN on day 3 *p.i.*, corresponding with peak MOG-QD signal. This is especially interesting because macrophages can engulf and present antigens to naïve T cells, which then become polarized towards regulatory or inflammatory phenotypes. Another antigen-presenting cell type, $CD11b^+$ dendritic cells, saw a steady increase over the one week study. Interestingly, a sustained drop in T cell frequency and increase in B cell frequency was seen in the draining LN beginning 3 days after injection. In future experiments, we will use this tool to investigate how antigen density and sequence affect biodistribution and immune functionality in both healthy and diseased mice. We also plan to exploit the theranostic capabilities of QDs by performing live, *in vivo* imaging experiments.

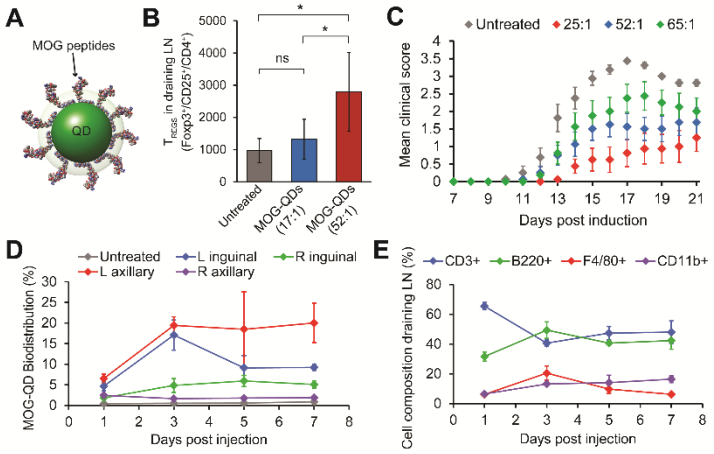


Figure 6 – MOG-decorated QDs (A) induce T_{REG} polarization in a self-antigen dose-dependent manner (B). Tolerance in EAE is correlated to MOG ligand density (C). Biodistribution of MOG-QDs (D) and the change in LN composition can be quantified (E).

SUSTAINED RELEASE VACCINE PLATFORMS FOR ENHANCED HUMORAL IMMUNITY

Gillie Agmon, Stanford University, Bioengineering
agmon@stanford.edu
Emily Gale, Stanford University, Biochemistry
Caitlin Maikawa, Stanford University, Bioengineering
Anthony Yu, Stanford University, Material Science & Engineering
Eric Appel, Stanford University, Material Science & Engineering

Key Words: Hydrogel, Immunomodulation, Supramolecular, Humoral Response

Vaccines aim to modulate the immune system to elicit a sufficiently robust, yet targeted, immune response with specifiable immune cell phenotypes.[1,2] Failure of a single vaccine administration to elicit such a response likely arises from inappropriate temporal control over antigen/adjuvant presentation and immune cell activation.[2] Recent work demonstrates the potential for sustained, low-level presentation of antigens and adjuvants for just over a week to yield more potent and long-lasting immunity.[3] However, an incomplete understanding of the enormously complex and dynamic immune system dramatically limits the ability to rationally design true, single-administration vaccines.[3] Our lab has developed injectable and self-healing polymer-nanoparticle (PNP) hydrogels with the ability to deliver multiple cargo of differing compositions.[4] Due to the supramolecular interactions between the HPMC-C_{12} polymer and PEG-PLA nanoparticles, these materials are prepared by simply mixing the components. This ability to streamline material preparation mitigates common challenges observed with manufacturing typical biomaterials at scale, thereby significantly enhancing translatability.[5] We have expanded this PNP hydrogel platform to allow for prolonged controlled release of an encapsulated model subunit vaccine composed of an antigen (ovalbumin) and adjuvant (poly(I:C)) (Figure 1A). The studies with this vaccine platform have yielded a deeper understanding of the effect of the kinetics of antigen/adjuvant presentation on immune activation. With a single injection of the vaccine, hydrogel formulations can elicit 10-fold higher antibody concentrations by day 35 compared to the same vaccine delivered in a typical bolus administration in PBS and had detectable antibodies past 120 days (Figure 1B). Upon an immune challenge, mice immunized with hydrogel-based vaccine formulations induced upwards of 3-fold higher antibody concentrations and higher affinity antibodies systemically. Cell phenotyping experiments indicate an increase in germinal center B cells in hydrogel formulations. These data, along with the boost in the humoral immune response, suggest that prolonged vaccine exposure from the PNP hydrogels leads to an increase in vaccine interaction with immune cells, allowing for somatic hypermutation and clonal selection of B cells. The unique properties of PNP hydrogels make this system ideally suited for controlling release of antigen and adjuvants to elucidate what molecular levers must be pulled, how hard, and when, to produce robust long-term immunity.

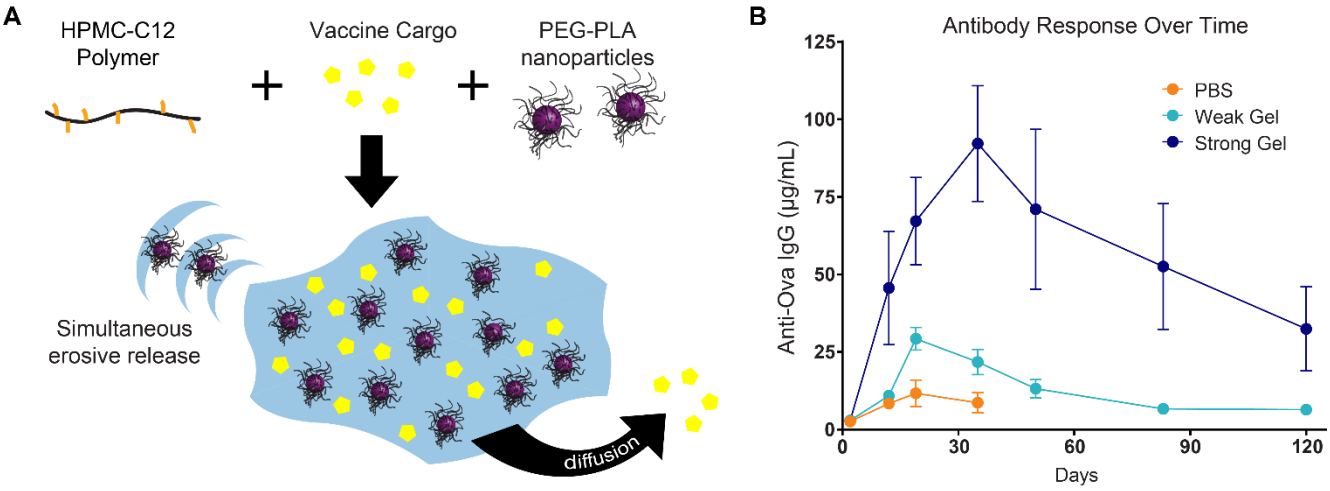


Figure 7 – (A) Schematic of the polymer-nanoparticle hydrogel with vaccine cargo. (B) Serum concentrations of anti-ovalbumin antibodies after single administration of vaccine.

[1] Swartz MA. Sci. Transl. Med. 2012;4. [2] Wen Y. Curr. Opin. Immunol. 2015;35:73. [3] Irvine DJ. Nature Mater. 2013;12:978. [4] Appel EA. Nat. Commun. 2015; 6:6295. [5] Yu AC. Proc. Nat. Acad. Sci. 2016;113