The NCI 60 human tumor cell line anticancer drug screen was developed in the late 1980’s as an in vitro drug discovery tool intended to supplant the use of transplantable mouse tumors in anticancer drug screening. Initially focused on lung cancer drug discovery, it rapidly became clear that some other cell types were necessary as controls. The finding that normal cell types available at the time, i.e. fibroblasts and certain epithelial cell populations such as renal epithelial cells, responded in vitro to anticancer drugs with extreme phenotypes (fibroblasts being pan-resistant and renal epithelial cells being pan-sensitive) in the assay selected for the screen, led to the use of other tumor cell lines as controls. Thus, panels of tumor cell lines were assembled, ultimately representing nine distinct tumor types. While the intent of the screen was to identify compounds with growth inhibitory or toxic effects to particular tumor types (the disease-oriented concept), the patterns of relative drug sensitivity and resistance generated with standard anticancer drugs were rapidly found to reflect mechanisms of drug action. The information-rich character of the screening data provided an additional and unexpected dimension to the screening model which has fueled development of powerful tools for database mining. Indeed, during more than a decade of use, the screen has produced a stream of unexpected discoveries that has impacted fields such as targeted anticancer therapy employing biological agents, virology, pathogenesis of bacterial toxins, potential, and molecular-targeted anticancer drug discovery. In this latter application, the NC160 profiles of new agents with distinct mechanisms emerging from novel screens can serve to define new mechanistic clusters or to decode previously enigmatic clusters in the existing database. In the case of engineered cell reporter screens, reference to the NC160 database can help define multiple mechanistic classes within active compounds identified in high-throughput screens.

In Vitro studies, immunity, and present new data on cellular resistance to viruses. In our talk I introduce eicosanoids, review our work on eicosanoid actions in insect immunity, and present new data on cellular resistance to viruses. In our In Vitro studies, Manduca sexta hemolymph was collected and diluted. The hemocytes were challenged with bacteria and microaggregation reactions increased in chal- lenger preparations. Treating the hemocyte preparations with pharmaceutical inhibitors of eicosanoid biosynthesis disabled the microaggregation reactions. The inhibition of the pharmaceuticals was reversed by treating the disabled cells with arachidonic acid or with PGE. We infer that hemocytes produce the eicosanoids responsible for mediating cellular immunity in insects. More recently, we considered eicosanoid actions in established insect cell lines. We used two cell lines, one non-permissive to viral infection (HzAM1, a pupal ovarian line from Helicoverpa zea) and one permissive (HzAM1, a pupal ovarian line from Heliothis vires- cens). Treating the HzAM1 line with inhibitors of eicosanoid biosynthesis, then challenging the cultures with the AcMNPV baculovirus, resulted in significantly increased proportions of cells producing virus and higher overall extracellular virus concentrations. We infer that eicosanoids affect one or more insect cellular mechanism(s) of viral resistance. We report on similar experiments with the permissive line in a poster presentation.
A-5
Experimental Approaches to Evaluation of Immune Functions in Mosquito Cell Lines. A. M. FALLOON, Department of Entomology, University of Minnesota, St. Paul, MN 55108. Email: fallo002@umn.edu

Expression of immune functions in vitro offers an approach to purifying antimicrobial gene products that protect an insect from infection and modulate host-pathogen interactions in vector insects. We have used the C7–10 cell line from Aedes albopictus and the Aag-2 line from Aedes aegypti to identify several classes of proteins that have antibiotic functions. Synthesis of antimicrobial products can be induced by exposing growing cells to heat-killed bacteria. The cells readily phagocytose dead bacteria, and exhibit associated physical changes including rounded morphology and aggregation. Biochemical changes include secretion of effector proteins, such as transferrin, cecropins, defensins and lysozyme, into the culture medium. Experimental approaches that have been particularly informative include analysis of radiolabeled proteins on polyacrylamide gels, purification of candidate peptides by HPLC, differential display and subtracted cDNA libraries, and protein analysis by tandem mass spectrometry. Our interests now focus on the interaction of the mosquito cell with the obligate intracellular bacterium, Wolbachia pipiens, and the means by which Wolbachia escapes the cellular immune response.

A-6
Determination of the Effects of Ecdysteroids and JH on Nodulation Responses. V. FRANSENS, G. Smaghe, G. Simonet, I. Claey, B. Breugelmans, A. De Loof, and J. Vanden Broeck. Laboratory of Developmental Physiology, Genomics and Proteomics, Department of Animal Physiology and Neurobiology, Zoological Institute, K.U. Leuven, Naamsestraat 59, B-3000 Leuven, BELGIUM and Laboratory of Agrozoology, Department of Crop Protection, Faculty of Bioscience Engineering, Ghent University, Coupure Links 653, B-9000 Ghent, BELGUIM. Email: vanessa.franssens@bio.kuleuven.be

Insects have a highly developed innate immune system, including humoral and cellular components. The cellular immune responses refer to hemocyte-mediated processes such as phagocytosis, nodulation, and encapsulation. Nodulation, which is considered the predominant defense reaction to infection in insects, is a complex process influenced by various endogenous factors. However, the precise mechanisms underlying nodulation remain largely unknown. In the present study, we examined the influence of the insect hormones 20-hydroxyecdysone (20E) and juvenile hormone (JH) on the laminarin-induced nodulation reaction in larvae of the flesh fly Neobellieria bullata. Treating third-instar larvae of Neobellieria bullata with 20E prior to laminarin injection enhanced the nodulation response in a dose-dependent manner. The edysone agonists RH2485, RH3849, and RH0345 similarly enhanced the nodulation reaction, although they were less active than 20E. In contrast to edysone stimulation, supplying larvae with JH or the juvenile hormone analogs (JHA), fenoxycarb and pyriproxyfen, significantly impaired their ability to form nodules in response to laminarin. These findings demonstrate for the first time that 20E and JH play an important regulatory role in the nodulation process.

A-7
Multipotent Adult Progenitor Cells for Vascular Repair. CATHERINE M. VERFAILLIE, Jeff Ross, Fernando Ulloa, Susan Keirstadt, and Aernout Luttun. Stem Cell Institute, University of Minnesota, McGuire Translational Research Facility, 2001–4th St SE, Mail Code 2873, Minneapolis, MN 55455. Email: verfa001@umn.edu

We and others have over the last 5 years demonstrated that cells can be cultured from bone marrow, blood or other tissues that have the ability to differentiate into multiple cell types, not only of mesoderm, but also endoderm and ectoderm. We termed these cells multipotent adult progenitor cells, whereas other groups have named these MIAMI cells, BSSCs, USSCs. We will present evidence that MAPCs can differentiate into endothelium, and be specified to arterial, venous or microvascular endothelium, as well as cells with functional and phenotypic attributes of vascular smooth muscle cells. As such MAPCs could be used to treat vascular insufficiencies. Approaches that will be presented include direct cellular transplantation and generation of bio-artificial vessels.

A-8
Cell Sourcing for Fibrin-Based Heart Valve-Equivalents. C. WILLIAMS, S. L. Johnson, P. S. Robinson, and R. T. Tranquillo. Department of Biomedical Engineering, University of Minnesota, Minneapolis, MN 55455. Email: tranquillo@ems.umn.edu

Native aortic valve leaflets are composed primarily of valve interstitial cells and myofibroblasts and lined with endothelial cells. However, dermal fibroblasts and smooth muscle cells are often used in tissue-engineered heart valves in attempts to reproduce the native architecture and composition in vitro. For example, we seek to fabricate a bioartificial valve by casting fibrin gel with entrapped cells in the geometry of a valve and controlling the cell-mediated remodeling of the fibrin. Porcine aortic valve cells (PAVCs) and human dermal fibroblasts (HDFs) express different phenotypic markers and perform distinct functions in vivo. In this study we compared PAVCs to HDFs in a fibrin remodeling assay using different basal culture media conditions (DMEM and DMEM/F12) supplemented with 10% fetal bovine serum, transforming growth factor beta (TGF), insulin, ascorbic acid (AA) and aminocaproic acid. We also studied the effect of two growth factors alone and combined on both cell types. Specifically, we compared TGF, fibroblast growth factor (FGF), and FGF with TGF supplementation of DMEM for HDFs and DMEM/F12 for PAVCs. Furthermore, we quantified the effect of different AA concentrations on collagen deposition and mechanical strength. PAVCs cultured in DMEM/F12 were provided with 0, 50 and 150 mg/ml AA and HDFs cultured in DMEM were supplied with 50 and 150 mg/ml AA. In both cases, AA was freshly prepared and added to the cultures times a week. Entrapped cells were cultured in fibrin gels and harvested at 3 and 5 weeks. Collagen and elastic deposition and cell phenotype were evaluated with light level histology and immunohistochemistry, and constructs were assayed for collagen, elastic, cellularity and mechanical properties. There were significant differences among samples cultured in different basal media for a given cell type and between cell types. Significantly greater collagen and elastic deposition and higher ultimate tensile strength compared to PAVCs. PAVC constructs without any AA supplementation contained less collagen compared to DMEM/F12. HDFs were able to compact the fibrin fibril network more extensively, deposit more collagen, and attain higher ultimate tensile strength and modulus were observed for HDF cultured in DMEM/F12 and constructs were assayed for collagen, elastic, cellularity and mechanical properties. There were significant differences among samples cultured in different basal media for a given cell type and between cell types. Significantly greater collagen and elastic deposition and higher ultimate tensile strength compared to PAVCs. PAVC constructs without any AA supplementation contained less collagen compared to the higher AA concentrations, but interestingly, there was no significant difference in collagen deposition between 50 and 150 mg/ml of AA. Replacing TGF with FGF in PAVC constructs caused excessive fibrin degradation and reduced deposition of new extracellular matrix. However, the opposite trend was observed in HDF constructs. These results underscore the importance of cell type and culture media selection and supplementation for the engineering of bioartificial heart valves based on remodeling of fibrin by entrapped cells.
Human bone marrow contains a population of mesenchymal stem cells (hMSC) capable of forming various types of mesenchymal tissues, including bone and cartilage. In vitro expansion and cultivation of hMSC on biomaterial scaffolds could facilitate osteochondral repair, where functional autologous cartilage/bone constructs would be grown and subsequently implanted into the defect site to promote healing. Bone-like and cartilaginous constructs have been made using hMSC and sponges made of biodegradable polyglycolic acid and gelatin. However, the scaffold size and composition were limited by poor mass transfer. We report here that a hydrodynamically active environment of rotating bioreactors markedly improved the structure and mechanical function of engineered cartilage and bone. Culture-expanded hMSCs were seeded onto porous silk scaffolds (8 mm diameter × 2 mm thick discs, 300–425 μm pores, >95% porosity; 5 × 10⁶ cells per disc). The resulting cell-polymer constructs were cultured for 5 weeks in rotating bioreactors in either control medium (DMEM with FBS), osteogenic medium (control medium with β-glycerophosphate, dexamethasone, ascorbic acid 2-phosphate, rhBMP-2) or chondrogenic medium (control medium with dexamethasone, ascorbic acid 2-phosphate, non-essential amino acids, TGF-β1, insulin). Constructs were assessed for wet weight (ww), dry weight (dw), confined-compression modulus (kPa), DNA, sulfated glycosaminoglycans (GAG), calcium (Ca), alkaline phosphatase activity (AP), histology (von Kossa and safranin-O), and imaged using µCT and contrast enhanced MRI. Bioreactor cultivation of hMSC on silk scaffolds yielded large (8 mm x 2 mm) bone-like tissue constructs with wet weight fraction of Ca approximately 2-fold higher than previously reported, and volume fraction of mineralized tissue within range of values measured for human lumbar vertebral bone. We assume that improved mass transfer in the rotating bioreactor as compared to static culture, in combination with osteogenic effects of BMP-2, were the decisive factors for this highly positive outcome for bone.
A-14
Cryopreservation of Hepatocytes: Role of Culture Configuration on Survival. ALLISON HUBEL. Department of Mechanical Engineering, University of Minnesota, 1100 Mechanical Engineering, 111 Church St. S.E., Minneapolis, MN, 55455. Email: hubel001@umn.edu

Hepatocytes are being studied for a wide variety of applications. Xenogenic hepatocytes are being used in liver assist devices for temporary liver support. Human hepatocyte transplantation is being used for a wide spectrum of liver diseases. Hepatocytes (human and animal species) are used extensively for in vitro studies of drug metabolism, metabolic and viral diseases of the liver. The supply of fresh differentiated hepatocytes is limited by the ability to induce proliferation of hepatocytes and effectively cryopreserve those cells. Thus, the ability to cryopreserve hepatocytes is essential to insuring supply of these cells. Two distinct challenges exist for preserving hepatocytes: preserving viability and differentiated function. Different approaches to improving post thaw recovery have been used: culture in specialized matrices and culture as spheroids. These approaches have also improved post thaw function as well. Other studies have shown that post thaw apoptosis is a significant factor in cell losses from freezing. Strategies to minimize these losses will be discussed. Finally, advances in our understanding of stem cells (adult and embryonic) may provide new sources of hepatocytes in the future. The importance of developing new sources of hepatocytes and effective methods of preserving them will also be important to the clinical and commercial application of these cells.

A-15
Biophysical and Molecular Changes Associated with Cryopreservation of Sperm. KEN ROBERTS. Medical School University of Minnesota, Departments of Urologic Surgery and Physiology, 6-125 Jackson Hall, 321 Church St. SE, Minneapolis, MN 55455. Email: rober040@umn.edu

The process of cryopreservation invariably induces biophysical changes in sperm, the severity of which depend on the cooling rate, cryoprotectant (CPA) used, and freeze-associated osmotic events. Many of these biophysical events affect the plasma membrane of the sperm and are ultimately dependent upon the plasma membrane’s permeability to water during freezing. These biophysical events induce cellular and molecular changes in the sperm, some of which manifest as capacitation-like changes which together have been referred to as ‘cryocapacitation’. Optimal cryopreservation parameters (freezing rates, CPA composition and addition, etc) serve to minimize these changes. It is possible that some of these cellular and molecular changes may be minimized, prevented, or reversed by modulation of sperm signaling pathways that lead to physiological capacitation. The biophysical and molecular events of freezing leading to cryocapacitation, and potential mechanisms for prevention of these events, will be discussed.

A-16
Thermal Injury Characterization for Biomedical Applications: In Vitro Model Systems. J. C. BISCHOF. University of Minnesota, Departments of Mechanical Engineering, Biomedical Engineering and Urology, 111 Church St. SE, Minneapolis, MN 55455. Email: bischof@umn.edu

Heat transfer plays a crucial role in many biomedical applications in cryobiology (biopreservation and cryosurgery) and hyperthermic biology (thermal therapies). In these applications, thermal excursions are used to selectively preserve or destroy cells and tissues. Biopreservation is an enabling technology to many biomedical fields including cell and tissue banking, cell therapeutics, tissue engineering, organ transplantation and assisted reproductive technologies. Thermal therapies including cryosurgery are increasingly important in all surgical sub-specialties for minimally invasive thermal destruction of tissues for cancer and cardiovascular disease treatment. In this talk work will be reviewed focusing on in vitro model systems to assess cellular and molecular phenomena that are important in defining outcomes of both cryobiological and hyperthermic biomedical applications. Model systems currently in use in our lab included: cell suspensions, monolayers, engineered tissues (fibrin and collagen based) as well as native tissue explants.

A-17
Three-dimensional Microenvironment and Breast Cancer Progression. PENNEY M. GILBERT1,2, Nastaran Zahir1,2, Matthew J. Paszek1,2, Jonathan N. Lakins1,2 and Kendice R. Johnson1,2. 1 Institute for Medicine and Engineering, UPENN, Philadelphia, PA, 19104; 2Department of Pathology, Research Institute, UPENN, Philadelphia, PA, 19104; 3Department of Biological Engineering, MIT, Cambridge, MA 02139; 4Abramson Family Cancer Research Institute, UPENN, Philadelphia, PA 19104; and 5GlaxoSmithKline, Philadelphia, PA, 19101. Email: pgilbert@mail.med.upenn.edu

Stromal-epithelial interactions drive development and maintain tissue homeostasis through a network of soluble and insoluble factors that operate within a three dimensional (3D) tissue. Genetic and epigenetic changes in mammary epithelial cells (MECs) cooperate with a modified tissue microenvironment to drive malignant transformation of the breast. We have been studying how an altered stromal microenvironment contributes to breast tumorigenesis and have specifically focused on understanding the role of changes in extracellular matrix (ECM) composition and organization and integrin expression and activity. Using immortalized normal, premalignant and tumorigenic MECs together with the 3D reconstituted basement membrane (rBM) morphogenesis assay and endothelial co-cultures we could show that the levels, type and activity of integrins change progressively and dramatically during MECs transformation. Recapitulating the altered integrin profile and activity of normal MECs could repress the malignant phenotype of the tumor cells, drive the premalignant behavior of the nonmalignant cells and promote the malignancy of premalignant MECs in culture and in vivo. We also found that tumor progression in vivo is associated with an incremental increase in matrix stiffness that is associated with an increase in integrin expression, focal adhesion maturation and signaling and that increasing ECM stiffness in culture is sufficient to drive similar tumor-like behaviors and alter integrin expression/activity. Accordingly, we have begun to explore how matrix-derived force could drive mammary tumorigenesis and understand what induces ECM stiffness. Interestingly, homeobox genes and force play critical roles in tissue development, and are frequently lost in tumors, and can regulate integrin and ECM expression. We showed that HoxA9 is lost in invasive human breast tumors and that re-expressing HoxA9 can revert the malignant phenotype of breast cancer cells in culture and in vivo coincident with BRCA1 induction and normalization of adhesion and integrin expression. Therefore, we demonstrate that proper regulation of the composition and dynamic interplay of cellular and ECM components is essential to the maintenance of tissue equilibrium and evasion of the malignant phenotype in the breast. (Support: DISS0402407, DODW81XWH-05-1-330, NCI CA078731 and DAMD17-01-1-0368).
A-18
3D In Vitro Models Reveal the Invasive, Drug Resistant Phenotype of Metastatic Melanoma. KEIRAN SMALLEY. The Wistar Institute, 3601 Spruce Street, Philadelphia, PA, 19104. Email: ksmalley@wistar.org
Anticancer drug discovery has been long hampered by the lack of predictive models. One explanation for this is that tumors are only drug resistant when grown in the correct tissue microenvironment. Clinically, melanoma is incredibly drug resistant, but this phenotype is rarely seen in tissue culture models. Mouse models, equivalent to human skin, have been difficult to develop due to the differences in anatomy and function of human versus mouse skin. In particular, melanocytes in human skin are aligned on the basement membrane and are dispersed among the epidermal keratinocytes. In mouse skin the melanocytes are situated deep in the hair follicles and dermis. In response to this, our laboratory has developed an organotypic model to recreate the architecture of human skin. However, while these models are histologically equivalent to human skin, they are time-consuming to grow and unsuitable for drug discovery studies. In response to this we have developed a simpler In Vitro model where melanoma cells are grown as spheroids and then implanted into collagen gels. Under these conditions the cells proliferate rapidly and invade into the surrounding collagen in a tumor stage-specific manner. Treatment of the melanoma spheroids with chemotherapy drugs revealed a more drug resistant phenotype than seen in 2D culture. More striking results were seen when the implanted melanoma spheroids were treated with inhibitors of pathways known to be active in melanoma. Cells derived from metastatic melanomas were completely resistant to inhibitors of the PI3/Akt, MEK/ERK and Src pathways when grown as implanted spheroids. However, this resistance was not seen when the same cell lines were grown in adherent 2D culture. The observed drug resistance was dependent upon the presence of serum and the implantation of the spheroids into collagen. Taken together these results demonstrate that culturing the metastatic melanoma cell lines under the correct microenvironmental conditions reveals their drug-resistant nature and that the tumor environment is a critical regulator of drug resistance.

A-19
Reconstructing and Deconstructing the Progression of Human Squamous Cell Carcinoma In 3D Tissue Models. ADDY ALT-HOLLAND. Tufts University, 55 Kneeland St., Room 116, Boston, MA 02111. Email: addy.alt.holland@tufts.edu
The construction of human 3D tissue models of epithelial tissues provides unique experimental paradigms that can trace the complex interplay between multiple cell and tissue types in a biologically-meaningful tissue context. These tissues provide a more global picture of how disease-associated pathways interact in an environment that mimics human tissues and serve as “surrogate” tissues that have set the stage for the accelerated translation of discoveries to the clinic through strategies that will allow target identification and validation. This presentation will describe how our laboratory has developed 3D tissue biology as a portal to discovery of pathways linked to human cancer progression and how these tissue models may serve as a paradigm for clinical translation in the future. We have developed tissue models that mimic distinct stages of squamous cell carcinoma (SCC) in humans including: 1—precancer, 2—low-grade carcinoma and 3—high-grade carcinoma. We have accomplished this by constructing 3D epithelial tissues at an air-liquid interface in which cells have been genetically-modified by suppressing expression of E-cadherin. In light of the emerging view that cancer is a disease of altered tissue architecture driven by abnormal interactions between tumor cells and their tissue microenvironment, we have studied the role of the tumor microenvironment in these stages of SCC progression. We have defined 4 distinct microenvironments that a potentially-malignant cell must encounter as it evolves from precancer to malignancy: 1—Intraepithelial dormancy, 2—Transepithelial migration through the epithelial layers, 3—Attachment to the basement membrane interface and 4—Degradation of basement membrane and stromal invasion. Tissue models that mimic these stages of progression will be described and mechanisms driving them will be outlined. By viewing each microenvironment as a target in the cancer progression pathway, these 3D models have great potential to help close the loop that exists between observations gleaned from rudimentary cell culture systems to those that may be applied in patients-care settings. If this occurs, we will we be able to fully realize the opportunity for translational discovery that these 3D tissue models provide us with.