Animal Posters

A-2000

Generation and Maintenance of Integration-free Human Induced Pluripotent Stem (hiPS) Cells from Peripheral Blood Mononuclear Cells in Serum- and Feeder-free Growth Factor Defined Medium. HIROTAKA NAKATAO¹, Sachiko Yamasaki¹, Eri Akagi¹, Atsuko Hamada¹, Manami Ohtaka², Ken Nishimura², Mahito Nakanishi², Shigeki Toratani¹, and Tetsuji Okamoto¹.


We previously reported the generation and maintenance of integration-free human induced pluripotent stem (hiPS) cells from Dental pulp cells (DPCs) in serum- and feeder-free growth factor defined medium with non-genome integrative Sendai virus vector (SeVdp) expressing Oct3/4, Sox2, Klf4, c-Myc. Compared to DPCs, peripheral blood mononuclear cells (PBMCs) are very attractive target cells for reprogramming because blood is routinely collected in the clinic. Here we report the efficient generation of iPSCs from PBMCs in serum-, and feeder-free conditions. PBMCs isolated from peripheral blood with the ficoll-hypaque density gradient method were cultured in RD6F serum-free medium supplemented with interleukin-2. After 6 d in culture, the cells were infected with SeVdp in hESF9 serum-free medium, which was originally developed for ES cell culture, with several extracellular matrix (type I collagen, gelatin, fibronectin, laminin) -coated dishes. The induction efficiency was evaluated by alkaline phosphatase staining. We successfully generated hiPSCs from PBMCs in serum-, and feeder-free conditions. PBMCs isolated from peripheral blood with the ficoll-hypaque density gradient method were cultured in RD6F serum-free medium supplemented with interleukin-2. After 6 d in culture, the cells were infected with SeVdp in hESF9 serum-free medium, which was originally developed for ES cell culture, with several extracellular matrix (type I collagen, gelatin, fibronectin, laminin) -coated dishes. The induction efficiency was evaluated by alkaline phosphatase staining. We successfully generated hiPSCs from PBMCs in serum-, and feeder-free conditions. PBMCs isolated from peripheral blood with the ficoll-hypaque density gradient method were cultured in RD6F serum-free medium supplemented with interleukin-2. After 6 d in culture, the cells were infected with SeVdp in hESF9 serum-free medium, which was originally developed for ES cell culture, with several extracellular matrix (type I collagen, gelatin, fibronectin, laminin) -coated dishes. The induction efficiency was evaluated by alkaline phosphatase staining. We successfully generated hiPSCs from PBMCs in serum-, and feeder-free conditions. PBMCs isolated from peripheral blood with the ficoll-hypaque density gradient method were cultured in RD6F serum-free medium supplemented with interleukin-2. After 6 d in culture, the cells were infected with SeVdp in hESF9 serum-free medium, which was originally developed for ES cell culture, with several extracellular matrix (type I collagen, gelatin, fibronectin, laminin) -coated dishes. The induction efficiency was evaluated by alkaline phosphatase staining. We successfully generated hiPSCs from PBMCs in serum-, and feeder-free conditions. PBMCs isolated from peripheral blood with the ficoll-hypaque density gradient method were cultured in RD6F serum-free medium supplemented with interleukin-2. After 6 d in culture, the cells were infected with SeVdp in hESF9 serum-free medium, which was originally developed for ES cell culture, with several extracellular matrix (type I collagen, gelatin, fibronectin, laminin) -coated dishes. The induction efficiency was evaluated by alkaline phosphatase staining. We successfully generated hiPSCs from PBMCs in serum-, and feeder-free conditions. PBMCs isolated from peripheral blood with the ficoll-hypaque density gradient method were cultured in RD6F serum-free medium supplemented with interleukin-2. After 6 d in culture, the cells were infected with SeVdp in hESF9 serum-free medium, which was originally developed for ES cell culture, with several extracellular matrix (type I collagen, gelatin, fibronectin, laminin) -coated dishes. The induction efficiency was evaluated by alkaline phosphatase staining. We successfully generated hiPSCs from PBMCs in serum-, and feeder-free conditions. These results suggest that our serum-, feeder- and integration-free system for both induction and culture of hiPSCs from PBMCs will open the door for safe and convenient explorations of the possibilities offered by regenerative medicine.

A-2001

Impairment of Corneal Wound Healing by Diclofenac is Verified in a Human Ex Vivo Front of the Eye Model with a Comparison to Rabbit. R. L. FISHER¹, J. E. Herrmann², J. Gao², and A. E. Vickers². ¹Vitron Inc., Tucson AZ and ²Allergan Inc., Irvine CA. Email: robyn@vitron.com

Diclofenac (DCF) administered topically post eye surgery to relieve pain and inflammation can delay corneal wound healing clinically. To characterize human versus rabbit response to DCF following eye injury, topical DCF (clinical formulation, 0.1%) was administered following an anterior keratectomy (AK) to: 1) NZW rabbit eyes in vivo, and on 2) enucleated rabbit and human eyes (Stern et al., Cornea 2006). Human eyes were procured with donor consent through Midwest Eye-Banks (Ann Arbor, MI) and the Lions Eye Institute for transplant and research (Tampa, FL). This novel ex vivo front of the eye model, inclusive of the anterior chamber, remained viable for several days in culture. Wound closure was quantified with fluorescein. The AK rabbit in vivo study revealed that DCF delayed wound (8 mm) healing, with a statistically significant delay of wound closure at 48 h (45%) and 72 h (64%) than time matched AK controls. In the ex vivo models, the initial wound size was set by the linear rate of closure in the AK untreated eyes. Administration of DCF significantly delayed the rate and area of wound closure in rabbit (2.5 mm) by 80% at 48 h and in human (8 mm) by 53% at 18 h. To further characterize DCF effects, gene expression profiles were evaluated in the ex vivo models by qRT-PCR. DCF treatment reduced the number of genes changed in rabbit (17%) and human (30%) compared to AK only. In rabbit cornea, DCF caused an up-regulation of cytoskeletal, remodeling and immune response genes and an absence of stress response genes. In human cornea, DCF suppressed many cytoskeleton and remodeling gene expression changes (collagen, growth factors and Timp1).
Injury Responses in Human Liver Slices to Clinical Doses of A-2002

To characterize and predict human response to drugs associated with liver adverse effects, via diverse mechanisms, an ex vivo human liver slice model was dosed daily for up to 3 d. Functional markers of antioxidant status, and gene expression profiles identified the cellular targets and pathways altered by drug exposure. Human liver tissue was obtained from procurement agencies, according to accepted medical and ethical standards, as outlined by the Uniform Anatomical Gift Act. All compounds were compared within each human liver at clinical concentrations linked with altered liver function. Some drugs are known to impact liver glutathione status and/or mitochondrial function: acetaminophen (APAP, 1 mM), methimazole (MMI, 500 μM), cyclosporin A (CSA, 10 μM), dantrolene (DTL, 10 μM), and etomoxir (ETM, 100 μM). Diclofenac (DCF, 1 mM) forms reactive intermediates and perturbs cell and mitochondrial function. Terbinafine (TBF, 100 μM) and carbamazepine (CBZ 100 μM) affect liver function, yet the mechanisms are not defined. Time-dependent differences in the functional markers of oxidative stress, liver slice ATP and GSH levels (24-72 h), supported the use of daily dosing to reveal persistent and statistically significant differences. Time-dependent differences in the functional markers of oxidative stress, liver slice ATP and GSH levels (24-72 h), supported the use of daily dosing to reveal persistent and statistically significant differences. To further characterize the compounds, gene expression pathways following 2-doses were compared. Ischemia induced cellular changes, which include mitochondrial dysfunction was evident for APAP, MMI, CSA, and DCF, as well as a stress response for APAP and DCF. Liver steatosis and cholestasis, as evident by effects on mitochondrial fatty acid and lipid metabolism, play a role with DTL and ETM. A progression of oxidative stress and an up-regulation of enzymes involved in glutathione metabolism are in common with TBF and CBZ. The viability of this ex vivo human liver slice model has successfully been extended to several days in culture. This has advanced its application to evaluate multiple dosing, which confirms doses that lead to drug-induced injury, of compounds intended for clinical use.

A-2003

Sex Differences in TFIIBB expression in C57BL/6 Mice Treated with EGCG. NICOLE NOLE, Jana Koo, and Laura Schramm. Department of Biological Sciences, St. John’s University, 8000 Utopia Parkway, Queens NY 11439. Email: nicole.nole05@stjohns.edu

RNA polymerase III (RNA pol III) is responsible for transcribing structural RNAs involved in cell processing (U6 snRNA) and translation (tRNA), creating a direct link between RNA pol III transcription and cell proliferation. TFIIB is required for accurate RNA pol III transcription. Deregulation of RNA pol III and TFIIB activity may be critical steps in tumor formation. BRF2, a subunit of TFIIB, has been identified as an oncogene in a squamous cell non small cell lung cancer. EGCG, a component of green tea, possesses anti-cancer properties and has in our lab demonstrated to inhibit RNA pol III transcription via the TFIIBB subunits in cervical cancer cells. However, to the best of our knowledge no one has investigated a role for EGCG in the regulation of RNA pol III transcription in lung cancer. Here we report that there are sex differences in the regulation of RNA pol III transcription by EGCG in adenocarcinomas (AC) of the lung, a subclass of non small cell lung cancer. H2347 female derived lung AC cells, treatment with EGCG significantly inhibits both gene internal and gene external RNA pol III transcription. In contrast, A549 male derived lung AC cells showed statistically significant elevation in RNA pol III transcripts when treated with EGCG. To elucidate a mechanism for the differential regulation of RNA pol III in lung AC, we looked at tumor suppressors and oncogenes which have been shown to regulate RNA pol III transcription in vitro. We show that HIC1 expression is statistically significant elevation in RNA pol III transcripts when treated with EGCG. H2347 female cell line could explain, in part, the observed decrease in RNA pol III transcription in female lung AC cells. We have not determined a mechanism for the stimulation of RNA pol III transcription in male lung AC. Thus, we sought to determine if sex differences in the regulation of RNA pol III by EGCG occurred in mice. In C57BL/6 mice we report that TFIIBB activity is differentially regulated by oral administration of EGCG.
Treating and preventing malaria has proven to be difficult in developing countries due to lack of infrastructure and economic hardship. Recently, research studying treatment using orally consumed dried leaves of *Artemisia annua* has shown promise. Previously we showed that various dietary constituents such as common fats and grains can alter artemisinin bioavailability when ingested with the dried leaves. Here we used the same simulated digestion method, to determine the effect of common protein-rich dietary constituents on artemisinin bioavailability and observed that digestion with two protein-rich dietary constituents, peanut butter and dried milk, decreased artemisinin bioavailability each by 33%. However, pure protein alone was not sufficient to reduce artemisinin bioavailability. In another earlier study using mice, oral treatment using *A. annua* dried leaves yielded 40 times more artemisinin in the blood of the mice than oral treatment with pure artemisinin. We used a Caco-2 model of the intestinal epithelium to determine if secondary metabolites found in *A. annua* increased artemisinin transport across the intestine and, thus, its bioavailability. Cells were grown in 12 well plates on cell culture inserts for 3 wk to allow for differentiation into mature enterocytes before experiments were performed. We observed that neither of the flavonoids, quercetin and rutin, altered transport of artemisinin across the intestinal border. Ongoing experiments are investigating the effect of other secondary metabolites, such as phenolic acids, at different concentrations and investigating the effect of other secondary metabolites, such as phenolic acids, at different concentrations and aim to elucidate their effects on the transport of artemisinin. These results will help explain the enhanced bioavailability of artemisinin when delivered from dried leaves vs. as the pure drug.

### A-2005

**TLR4/CD14 and DNMT1 in Liver Biopsied Tissue and Differentiated Hepatocyte-like Cells. MATTHEW G. BANGE**, 1, Joshua B Alley2, Laura Fitzgerald2, Sanjay Samy2, Michael L. Shuler1, and Magnolia Ariza-Nieto1. 1Department of Biomedical Engineering, Cornell University, Ithaca, NY and 2Guthrie Clinic, Sayre, PA. Email: mgb226@cornell.edu

Our group is developing a human in vitro model to study epigenetic disregulatory states. Extracellular membrane vesicles are thought to participate in cell to cell communication by containing microRNA and transferring it between cells. To establish a vesicle biomarker, clinical samples from patients who had undergone Roux-en-Y gastric bypass were analyzed using qPCR to measure levels of CD14, a vesicle membrane protein and cofactor in the TLR super family. A significant negative correlation was found between CD14 and DNMT1 ($\rho = -0.5501$, $P = 0.0180$, $n=18$). This research was done as part of the IRB approved clinical trial GHS #1207-27. Using hepatocyte-like differentiated mesenchymal stem cells from the patients, we will attempt to replicate the clinical observations in vitro. MSCs are grown to confluence then differentiated into hepatocyte like cells following Stock’s differentiation protocol (P. Stock, 2010). The cells are then characterized by qPCR using GAPDH as the housekeeping reference. The hepatocyte like cells are treated with pharmacological compounds like 5-azacytidine to modulate the expression of DNMT1 in vitro. As far as we know, this is the first report associating liver CD14 and DNMT1 in humans as well as using MSC derived hepatocyte-like cells to replicate clinical observations in vitro.

### A-2006

**Synthetic Bone Scaffolding for Tissue Engineering: Development of Coating to Improve Mechanical Strength of Mg Alloy/DCPD Composite. ASTER G. SAMUEL, Nida Tanataweethum, and Tien-Min Gabriel Chu*. IUPUI School of Dentistry, Department of Orthopaedic Surgery, Indianapolis, IN. Email: astersamuel@gmail.com, tgchu@iupui.edu (Corresponding author)**

The development of tissue engineering and regeneration constitutes a new platform for translational medical research. Effective therapies for bone engineering employ the coordinated manipulation of cells, biologically active signaling molecules, and biomimetic, biodegradable scaffolds. Dicalcium phosphate dihydrate (DCPD) scaffolding cement composites have many desirable properties for bone tissue engineering, including their excellent biocompatibility, biodegradability, and resorbability. However, DCPD cements are intrinsically weak and brittle and such traits are undesirable. To address these limitations, in this study we employed the addition of sodium citrate as a regulator and polylactic acid (PLLA) as a reinforcing agent by developing a 5% composite PLLA coated Mg alloy WE43-B/DCPD with enhanced mechanical strength and modulus to characterize the phase composition of Mg alloy WE43-B/DCPD. We synthesized DCPD cements with a 1:1 ratio of monocalcium phosphate monohydrate and beta-tricalcium phosphate. We prepared magnesium alloy/DCPD composite samples by mixing 5% wt and 10 % wt of magnesium alloy with the calcium phosphate powders. The cement paste were prepared by mixing the powders with 100 mM sodium citrate in 15% v/v glycerol at powder to liquid weight ratio $P/L = 1.50$. To fabricate the PLLA/Mg alloy/DCPD composite samples, Mg alloy/DCPD composite were coated with 5% wt of PLLA. The phase composition of the samples were characterized by x-ray diffraction investigations. The modulus and compressive strength of the samples were...
also measured. We showed that PLLA coating improved the compressive strength of the DCPD group, 5% Mg alloy/DCPD group and 10% Mg alloy/DCPD group by 2.47, 1.56, and 1.30, respectively. Based on this investigation, we conclude that a combination of template-casting and polymer incorporation methods can be applied to fabricate a cement/polymer biodegradable scaffold for bone tissue regeneration with significantly improved the mechanical properties of the Mg alloy/DCPD composite.

A-2007

Tackling the EML4/ALK Fusion Oncogenes in Murine Models. J. HAWES, T. Vilimas, K. Collins, and S. Kozlov. Center for Advanced Preclinical Research (CAPR), SAIC-Frederick, Inc., Frederick National Laboratory for Cancer Research (FNLCR), Frederick MD 21702. Email: jaden.hawes@gmail.com, Corresponding Author: kozlovse@mail.nih.gov

Non-small cell lung cancers (NSCLC) expressing the EML4/ALK fusion protein are frequently resistant to current treatment strategies and lead to relapse. The molecular mechanisms of EML4/ALK tumorigenesis and resistance are not well understood. EML4/ALK transgenic mouse lines were screened for germline transmission and inducible expression of the EML4/ALK fusion protein. Mouse embryonic fibroblasts (MEFs) were isolated from mice harboring an EML4/ALK transgene and induced to express the EML4/ALK fusion protein in vitro by exposing the cells to doxycycline. After identification of a functional transgenic line, mice were exposed to doxycycline in vivo and lung tissues were examined microscopically. Induced expression of the EML4/ALK fusion protein in transgenic EML4/ALK mice was sufficient to induce lung hyperplasia, the first step in lung tumorigenesis. This data indicates that EML4/ALK transgenic mice may be useful as a murine model of EML4/ALK lung tumorigenesis. This data indicates that EML4/ALK transgenic mice may be useful as a murine model of EML4/ALK lung tumorigenesis. This data indicates that EML4/ALK transgenic mice may be useful as a murine model of EML4/ALK lung tumorigenesis. This data indicates that EML4/ALK transgenic mice may be useful as a murine model of EML4/ALK lung tumorigenesis.

A-2008

Comparative Study of Icosahedral Viruses by Nanoparticle Tracking Analysis, DLS and TEM. N. A. NIKITIN, E. A. Trifonova, E. G. Evtushenko, E. K. Petrova, J. G. Atabekov, and O. V. Karpova. Lomonosov Moscow State University, 1/12 Leninskie Gory, 119991, RUSSIA. Email: nikitin@mail.bio.msu.ru

The quantification of virus titer and characterization of viruses and virus-like particles in terms of size homogeneity and aggregation state are very important in development and standardization of vaccines as well as other biotechnological applications. Widely used techniques, such as transmission electron microscopy (TEM) involve fixation and drying of the viromes on the substrate. Thus aggregation state and minor contaminations with adventitious particles couldn’t be revealed with TEM. Dynamic light scattering (DLS) provides information about size in liquid, but with low resolution, and it’s too sensitive to contaminations. Novel method of Nanoparticle Tracking Analysis (NTA) provides supplementary information about the viromes size with high resolution, titer and aggregation state in liquid. The method calculates the equivalent spherical diameter of viral particles, thus size characteristics is most appropriate primarily for icosahedral viruses. Animal icosahedral virus (encephalomyocarditis virus) and plant icosahedral viruses (cauliflower mosaic virus, brome mosaic virus, bean mild mosaic virus) were analyzed by NTA. The data were compared with DLS solution measurements and TEM. Experiments were carried out under different conditions (ionic strength, pH, storage time). The detection limit of 30 nm for correct sizing of small spherical viruses by NTA was determined. Virus particles of this size are readily visualized by NTA, but measured size is slightly overestimated even with 405 nm laser used. The closest values of viruses characteristics obtained by NTA, DLS and TEM were in the storage buffers with freshly purified/thawed samples. The work was funded by the Russian Science Foundation (grant 14-24-00007).

A-2009

The Novel Candidate Vaccine Against Rotavirus Infection Based on Structural Modified Tobacco Mosaic Virus. E. A. TRIFONOVA, N. A. Nikitin, E. K. Petrova, O. V. Karpova, and J. G. Atabekov. Lomonosov Moscow State University, Dept. of Virology, 1-12 Leninskie Gory, 119991, RUSSIA. Email: trifonova@mail.bio.msu.ru

Rotavirus infection is the leading cause of infant severe diarrhea all over the world. Rotavirus is also one of the main causes of children deaths in the first year of life. Plant viruses and their virus-like particles are promising platforms for candidate vaccines development. The main advantage of plants viruses is their biosafety because plants and animals have no common pathogens. The present work is focused on the design of a new candidate vaccine against rotavirus. The novel vaccine consists of structural modified tobacco mosaic virus and recombinant rotavirus antigen. We have previously shown that thermal structural remodeling of tobacco mosaic virus leads to transformation of rod-like virions into spherical particles (SPs). The size of SPs depends on initial virus
concentration and could vary from 50 nm to 1-2 μm. SPs are very stable to external factors, biosafe, RNA-free, highly immunogenic and have unique adsorption properties. We have previously demonstrated that SPs could be an effective adjuvant. SPs boost the immune response to the model antigens. In the present work the complex of SP with recombinant antigen containing epitope of rotavirus VP6 protein was obtained. VP6 is a structural protein of interior virus capsid and has high antigenic properties. The formation of “SP-rotavirus antigen” complex was detected by method of immunofluorescence microscopy. The ability of rotavirus antigen adsorbed on the SP surface to specifically react with commercial virus antiserum was shown. We have obtained preliminarily data that SPs enhance the immune response on rotavirus antigen. “SP-rotavirus antigen” complexes are the novel type of candidate vaccine against rotavirus infection. The work was funded by the Russian Science Foundation (grant: 14-24-00007).