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# Animal Posters

## A-2000

Cell Matrix Interactions in 3D Alginate Cell Culture System. THERESE ANDERSEN, Christine Markussen, and Michael Dornish. FMC BioPolymer/NovaMatrix Industriveien 33, N-1337 Sandvika, NORWAY. Email: therese.andersen@fmc.com, michael.dornish@fmc.com

NovaMatrix®-3D is an alginate-based cell culture system comprising an alginate foam matrix and an alginate immobilizing solution. Cells are entrapped throughout the alginate foam by first suspending them in a solution of sodium alginate that is applied to the foam. As the foam absorbs the suspension, in situ gelation occurs as calcium ions are donated from the foam cross-linking the added alginate. A hydrogel forms in the pores of the foam entrapping the cells. Some cells, however, require signaling molecules and matrix interaction in order to proliferate. This was evaluated by culturing cells in the presence of alginate to which the peptide sequence RGD (Arg-Gly-Asp) was covalently attached (RGD-alginate) thereby facilitating a signaling cascade via integrin-RGD interaction. Single cells of NHIK 3025 (human cervix carcinoma), OVCAR-3 (human ovarian adenocarcinoma), SKOV-3 (human ovarian carcinoma), NIH:3T3 (murine fibroblasts), C2C12 (murine myoblasts), V79-379A (hamster lung fibroblast) and MDCK (Madin Darby canine kidney) suspended in 0.5 - 1 % alginate or RGD-alginate were added to NovaMatrix®-3D foams. Cell localization within the foam was visualized by confocal microscopy of fluorescently labeled cells. Cell proliferation was measured by counting cells after de-gelling the foam using sodium citrate. Similar cell proliferation and spheroid formation occurred during three wk of culture in foams with or without RGD-alginate for NHIK 3025, SKOV-3, 3T3 and V79-379A indicating that these cell lines do not require the presence of RGD within the alginate matrix for cell growth. However, during the same period, cell proliferation of OVCAR-3, MDCK or C2C12 was only seen in the foams containing RGD-alginate. The proliferation rate was affected by the RGD density. Cells that did not proliferate in alginate without RGD were still metabolically viable. Cell-matrix interaction can be controlled by type and concentration of signaling molecules covalently attached to the alginate. Portions of this work were funded by METOXIA project no.222741, EU 7th Framework Programme.

## A-2001

Increased Viability of Encapsulated Cells for Sensing Applications. B. C. BUTLER[1], K. Moler[1], H. Roach[2], K. Dierksen[2], and J. Trempy[2]. [1]Luna Innovations, Inc., Charlottesville, VA 22903 and [2]Oregon State University, Corvallis, OR 97331. Email: butlerb@lunainnovations.com

Living cell–based sensors have proven effective in a variety of detection applications. These sensors exploit the native signaling cascades inherent in living cells to amplify specific signals by many orders of magnitude. However, cell line–based sensors require frequent replenishment with new cells or media regeneration due to the sensitivity of the cells to the ex-vivo environment and intrinsic factors associated with cell growth and metabolism. These restrictions have limited the application of these living cell-based sensors to lab-based detection applications. Creating practical cell-based sensors for field deployable detection systems requires a biocompatible interface between the cells and the macro world. Specifically, the incorporation of a functional bio-inorganic interface that allows cellular interaction at the nanoscale while still maintaining viability. To meet this need, Luna Innovations has employed encapsulation techniques to immobilize a variety of cell lines, demonstrating an extended operational cellular lifespan while also permitting ready access to toxic or pathogenic agents in the environment and retaining specific signal recognition for encapsulated cells.

## A-2002

Prospect of Neural Cells Derived from Human Pluripotent Stem Cells for Application of In Vitro Developmental Toxicity Test. Sumiyo Mimura[1,2], Mika Suga[1], Masaki Kinehara[1], Daiki Tateyama[1], Mitsuhi Hirata[1], Hiroki Nikawa[2], Kana Yanagihara[1], and MIHO KUSUDA FURUE[1]. [1]Laboratory of Cell Cultures, Department of Disease Bioresources, National Institute of Biomedical Inovation, Osaka, JAPAN and [2]Department of Oral Biology & Engineering, Division of Oral Health Sciences, Graduate School of Biomedical Sciences, Hiroshima University, Hiroshima, JAPAN. Email: mkfurue@nibio.go.jp

A medication which a mother may have taken during her pregnancy, in some cases causes drug-induced congenital

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diseases. However, there is no reliable screening test that can predict its developmental toxicity. Understanding the molecular mechanisms underlying neural development would encourage the development of alternative in vitro embryo-toxicity tests and contribute to a decreased incidence of drug-induced congenital diseases. Using a growth factor defined serum- and Xeno- free hESF-FX medium, in adherent monolayer culture, we developed a protocol to promote human pluripotent stem cell differentiation into neural cell lineage. In the culture conditions, the effect of exogenous factors can be analyzed without confounding influences of undefined components. This protocol could be used to help clarify the mechanisms underlying neural development and to assist the development of in vitro alternative developmental toxicity test for drug-induced congenital disease.

**A-2003**

A Novel Molecular Tool Box for Fish Cell Analyses. MARINA GEBERT, Julia Kirchhof, Sebastian Rakers, and Charli Kruse. Fraunhofer Research Institution for Marine Biotechnology, Paul-Ehrlich-Str. 1–3, 23562 Luebeck, GERMANY. Email: marina.gebert@emb.fraunhofer.de

The number of novel fish cell cultures from different species was growing with an amazing rate in the last years. Their applications are ranging from replication and analyses of fish viruses, (eco-)toxicology studies, immunology, biomedical sciences, biotechnology and aquaculture, just to name a few. However, characterization of the gene and protein expression profile of a mixed cell population derived from a whole organ and present in culture is often intermittent. Although molecular and genetic tools like primers or antibodies as well as other markers are used in a wide variety of biological, pharmaceutical and medical applications, they are just beginning to be implemented in the field of aquatic sciences. Information about genomes and proteomes in most fish species- excluding zebrafish and few others- is still scarce and thus analyzing these molecules often is labour-intensive and includes steps like gene sequencing or production of antibodies. Fortunately, a shortcut on the long way of sequencing can be used for single genes by aligning homologous genes from other species and developing degenerated primers. By this, genes from aquatic species with yet unknown sequence can be amplified. Similarly, many homologous proteins of mammals and fishes share high similarities. Antibodies directed against mamalian proteins can thus also be used for the detection of their equivalents in fishes. Here, we present a method for the development of primers for yet unknown genes as well as a comprehensive list of suitable cross-reactive antibodies for the analyses of fish cell cultures.

**A-2004**

Selectively Expressed Heat Shock Protein 70 Prevents Diet-induced Changes in Pancreatic Structure and Function. M. CIANCIO[1,2], S. Duminie[3], M. Manalastas[1], C. Evans[4], and K. LePard[2]. [1]Biomedical Sciences Program, [2]Physiology Department, [3]NIH-NIDDK Step-up Program, and [4]Physical Therapy Department, Midwestern University, Downers Grove, IL 60515 Email: mcianc@midwestern.edu

Obesity is linked to poor dietary habits, lack of exercise (EX), and morphological and functional changes in the pancreas. Our laboratory previously demonstrated that voluntary EX suppressed high fat (HF) diet induced pancreatic changes. EX significantly induced pancreatic heat shock protein 70 (Hsp70) in the HF fed mice compared to sedentary (Sed) controls [Sed (n=5), 1,168±88; Ex (n=4), 1,929 ±57* pg/mg protein; *p<0.05], which significantly correlated with improved glycemic control (r=−0.608, p<.05). We hypothesized that EX-independent Hsp70 would prevent HF-induced pancreatic changes. Pancreatic structure and function were determined after 16 wk on a HF diet using 4 Hsp70 transgenic (TG) mice that selectively express Hsp70 (Sed n=2; EX n=2) and 8 wild-type (Sed n=4; Ex n=4) mice. TG mice had significant pancreatic Hsp70 levels (3,162 pg/mg protein, n=2) without EX. Islet perimeters (Per), maximum (max) and minimum (min) diameters (dia), and areas (A) were measured from digital pancreatic images (13–50 per mouse) using Image ProPlus. Measurements were log transformed and statistically compared by 2-way ANOVA (factors: WT or TG and Sed or EX) using SPSS to determine significant main effects and interactions. Morphological parameters were significantly decreased by 20-40 % in TG mice compared to WT mice [Main effect: F (1,458), p<0.05; Per=7.254; max dia=7.553; min dia= 14.489; A=10.602]. Ex significantly reduced min dia by 18 % [Main effect: F (1,458)=4.677] with a trend to reduce A [Main effect: F (1,458)=3.565; p=0.06]. No interactions were observed for any morphological parameter [WT/ TG*Sed/Ex: F (1,458), p>0.05; Per=0.942; max dia= 1.018; min dia=0.299; A=0.698]. Oral glucose tolerance was improved in TG compared to WT mice [area under the curve (mean ± SD): WT (n=14), 38,672±10,468; TG (n=4), 21,409±2,165*; Main effect: F (1,14)=15.923; *p<0.05]. Selective Hsp70 prevented HF induced islet hypertrophy and improved glycemic control, suggesting that Ex-induced pancreatic Hsp70 may be an important mechanism to maintain islet morphology and function.

**A-2005**

Applications of Fish Cell Culturing to Conservation Biology: A Look at *Oreochromis esculentus* Cell Cultures for Genome

Resource Banks. M. FILICE[1,2] and G. F. Mastromonaco[1,2]. [1]Toronto Zoo, 361 Old Finch Ave, Scarborough, ON, M1B 5K7, CANADA and [2]University of Guelph, 50 Stone Rd E, Guelph, ON, N1G 2W1, CANADA. Email: mfilice@uoguelph.ca

The global decline of fishes has promoted efforts to systematically bank gametes, embryos, and cells into genome resource banks (GRBs). GRBs are important tools in conservation because they preserve valuable genotypes and maintain high levels of genetic diversity. Establishing somatic cell GRBs enables the banking of genetic material from animals that are post- or non-reproductive preserves the entire genome of the individual and is easier to implement in both the laboratory and the field. Although GRBs are important, there is little knowledge about the effects of long-term culture and cryopreservation on the viability of fish cells for nuclear transfer. To examine the effects of long-term culturing and storage on fish cells the ngege (*Oreochromis esculentus*) was chosen as the model fish. The ngege is an African cichlid classified by the International Union for Conservation of Nature and Natural Resources (IUCN) as critically endangered. The IUCN Species Survival Plan insists that ngege be maintained in captive breeding programs across North America prior to their release in Lake Victoria. In captivity, ngege experience rapid morphological and behavioural changes. For this reason, the IUCN Freshwater Fishes Taxon Advisory Group wants to develop regional cell culture banks of unaltered fish founders. To establish this goal, ngege caudal fins will be clipped, and cultured in L-15 media supplemented with 10 % fetal bovine serum. Culture quality will be assessed microscopically by determining chromosome normality, number of population doublings until senescence, cell viability of donor explants, and cell proliferation. Data obtained from this study will provide the foundation for global fish conservation through the use of GRBs and assisted reproductive technologies.

**A-2006**

Cryopreservation of Human Epidermal Skin Models. L. H. CAMPBELL, Z. Chen, H. Kershaw, and K. G. M. Brockbank. Cell & Tissue Systems, Inc. North Charleston, SC 29406. Email: lcampbell@celltissuesystems.com

In an effort to reduce the use of animals for toxicity testing, companies have developed in vitro skin models or equivalents to replace the Draize skin irritation test. This development has been accelerated by a European Union regulation that prohibited the use of animals for collecting toxicological data on cosmetic ingredients that began in 2009. Presently, these constructs are made to order and require a lead time of several weeks before they can be used. Thus, the ability to cryopreserve these constructs would permit banking, providing the end-user greater flexibility regarding the execution of experiments while providing the supplier the opportunity to perform quality control evaluations to insure the quality and consistency of the construct. In this study adherent human keratinocytes and the construct Epiderm™, supplied by the Mattek Corp, were used to develop a cryopreservation protocol for these skin constructs. While seemingly straightforward, these constructs are produced in well inserts and so the complexity of an insert in a multiwell plate provided some challenges for the development of a successful cryopreservation scheme. Initial cryopreservation protocols were assessed using adherent keratinocytes. Several cryopreservation solutions and cooling profiles were then evaluated demonstrating cryopreservation of these constructs. Interestingly, while many, including us, have successfully cryopreserved suspended keratinocytes in dimethyl sulfoxide (DMSO), neither adherent keratinocytes on plastic culture substrates nor the constructs demonstrated significant cell viability after cryopreservation with DMSO. In fact, very little if any viability was observed when 10 % DMSO is used. However, solution formulations containing up to 15 % glycerol demonstrate improved viability after thawing. These more successful formulations are similar to those employed for skin allograft preservation for use in burn patients. Experiments are in progress to further optimize cryopreservation of tissue engineered skin equivalent constructs.

**A-2008**

Establishment of Transgenic Cell Line for Expressing Human Alpha-lactalbumin. S. X. CAO, X. J. Feng, and C. H. Meng. Institute of Animal Science, Jiangsu Academy of Agricultural Sciences, Nanjing, Jiangsu 210014, CHINA. Email: caoshaoxian@163.com

The combination of somatic cell nuclear transfer (SCNT) and transgenic technology leads to the production of transgenic cloned animals, where in the preparation of competent transgenic donor cells is the pivotal upstream step. The objective was to establish an effective procedure to prepare human alpha lactalbumin(hα-LA)transgenic donor cells to produce hα-LA protein in the dairy goat mammary gland. Thus, a mammary-specific expression vector for hα-LA gene was constructed. Firstly, 5.5 kb 5′β-lactoglobulin (βLG) containing promoter was cloned and PSSC-9-βLG – EGFP was constructed and transfected into goat mammary epithelial cells (GMEC) for promoter bioactivity analysis in vitro. Next, a mammary-specific expression vector(5A) haboring neo and EGFP dual markers was constructed for expression of hα-LA. Then it was transfected into GMEC by Lipofectamine™LTX and PLUS™ Reagents, and induced by hormonal signals. It's mRNA transcript was correctly

expressed in transfected GMEC cells and hα-LA protein was detected in the supernatant of transfected GMEC by Western blotting. The hα-LA gene vector 5A was then introduced into fetal fibroblast (from dairy goats) to prepare competent transgenic cells. Reliable hα-LA transgenic fibroblast cell clones from a single round of transfection were isolated by 96-well cell culture plates and screened with nested-PCR amplification and EGFP fluorescence. The growth curve of transgenic cells ,the EGFP fluorescence and adherence rate showed that it can be used as donor cells after freezing 6 mo. This study may provide an effective upstream system to prepare SCNT donor cells for the production of human recombinant protein from the milk of transgenic animals.

**A-2010**

Efficacy of Platelet Antimicrobial Peptides Produced by Nanosecond Pulse Activated Platelets Against Opportunistic Bacteria. LOREE C. HELLER[1,2], Chelsea M. Edelblute[1], Yeong-Jer Chen[1], Richard Heller[1,2], and Barbara Hargrave[1,3]. [1]Frank Reidy Research Center for Bioelectrics; [2]School of Medical and Laboratory Radiation Sciences; and [3]Department of Biological Sciences, Old Dominion University, Norfolk, VA. Email: lheller@odu.edu

*Acinetobacter baumannii*, *Pseudomonas aeruginosa*, and *Staphylococcus aureus* are medically important opportunistic pathogens. Multi-drug resistance is becoming more common in these and other clinically relevant bacteria, requiring the development of new antimicrobial methods. First described in 1976 in *Drosophila*, many organisms are now known to produce antimicrobial peptides (AMPs). These peptides vary significantly in amino acid sequence, although the same fundamental amphipathic helical structure is maintained. In mammals, AMPs are produced by epithelial and myeloid cells, including platelets. Activated platelets secrete the content of their granules. More than 30 peptides with a variety of functions are released from the α granules, including a number of AMPs. One of the many natural platelet activators is thrombin; bovine thrombin is used clinically to form platelet gel. Platelet aggregation can also be induced by the application of nanosecond pulsed electrical fields (nsPEFs). The clinical use of this pulse activation may avoid side effects associated with the use of a bovine protein. In this study, the antibacterial efficacy of AMPs secreted by porcine and human platelets activated by nsPEFs and thrombin was compared using minimum inhibitory and bactericidal concentration quantification. The potential in vivo antibacterial efficacy of platelet gel was tested in an in vitro porcine skin model. Significant inactivation of opportunistic bacterial pathogens was observed. While the method of platelet activation varied, the subsequent antibacterial efficacy was dependent, as expected, on the level of platelet degranulation.

**A-2014**

Improved Longevity in an Impedance Based Toxicity Sensor. T. M. CURTIS, T. Thorn, and A. Howard. Department of Biological Sciences, State University of New York at Cortland, Cortland, NY 13045. Email: Theresa.curtis@cortland.edu

Previous work has shown that using ECIS (electric cell-substrate impedance sensing) to monitor the impedance of a variety of vertebrate cell monolayers provides a sensitive measure of toxicity for a wide range of chemical toxicants. One major limitation to using this system for chemical toxicant detection in the field is the difficulty of maintaining cell viability over extended periods of time with minimal support until the cells are needed for testing. The present study was performed to identify a cell line that would allow ECIS based toxicity sensing in true field conditions. A variety of invertebrate and vertebrate cells were screened for their ability to form a cell monolayer on the ECIS electrodes that was stable for extended periods of time. The invertebrate cell lines screened were unable to form stable monolayers on the ECIS electrodes that yielded high impedance readings needed for toxicity testing. Two of the vertebrate cell lines screened exhibited favorable portability characteristics on the ECIS electrodes. Mouse sertoli cells exhibited high impedance values for extended periods of time at room temperature without exogenously added $CO_2$. Gecko lung epithelial cells yielded stable high impedance values at a wide range of temperatures without exogenously added $CO_2$. Future work with these cells lines will characterize their sensitivity to a wide range of chemical toxicants to determine if they are good candidates for use in a portable ECIS based toxicity sensor.

**A-2016**

Replication of Very Virulent Infectious Bursal Disease Virus in the Chick Embryo Chorioallantoic Membrane Cells. M. H. MOHAMMED, M. Hair-Bejo, A. R. Omar, and I. Aini. Faculty of Veterinary Medicine, Universiti Putra Malaysia, 43400 UPM Serdang, Selangor, MALAYSIA. Email: majed_mohammed@putra.up.edu.my

The susceptibility of the chick embryo chorioallantoic cells to very virulent infectious bursal disease (vvIBDV) was characterized after five consecutive passages in chick embryo chorioallantoic cells. Virus replication was monitored by cytopathic effect observation, indirect immunoperoxidase, and reverse transcription polymerase chain reaction (RT-PCR). After 48 h post-infection (pi) at first passage, the cytopathic effect was characterized by rounding up of cells and monolayer detachment, intracytoplasmic brownish colouration was readily observed by from 24 h p.i onwards, viral RNA from vvIBDV-infected monolayers was

demonstrated by RT-PCR. Tissue culture effective dose50 ($TID_{50}$) was used to measure virus titration performed on chick embryo chorioallantoic cells, and the titer in second passage was $10^{6.6}$ $TID_{50}$/ml. The results obtained in this study suggested that the chick embryo chorioallantoic cells can be used for vvIBDV propagation, and this may trigger new aspects for tissue culture adaptation of such fastidious virus in the future.

**A-2018**

The Establishment and Potential Uses of a Yellow Perch (*Perca flavescens*) Cell Line**.** K. SPITERI[1], N. T. K .Vo[2], C. Way[1], and L. E. J. Lee[1]. [1]Department of Biology, Wilfrid Laurier University, Waterloo, ON, CANADA N2L 3C5 and [2]Department of Biology, University of Waterloo, Waterloo, ON, CANADA N2L 3C5. Email: spit2570@mylaurier.ca, llee@wlu.ca

Yellow Perch (*Perca flavescens*) is an economically important species that sustains a multimillion dollar industry in the Great Lakes commercial fisheries and aquaculture as well as in sports fisheries. The value of perch in the Canadian market has ranged between 10 to 15 dollars/lb retail, thus it is a highly valued fish. The recent reports of Viral Hemorrhagic Septicemia incidence in the Great Lakes, as well as reports of microsporidian infections with Heterosporis species affecting Yellow Perch is threatening the viability of this fisheries and there is a major need to develop cell lines from these species. Being indigenous species to Canada and USA, Yellow perch have also been used as models for toxicological and ecotoxicological applications and there is a demand for in vitro models. Wild caught adult Yellow perch from Pigeon Lake near Peterborough, Ontario, were killed on site by amateur fishermen, kept on ice for 2–3 d before being donated for tissue processing. Despite the long post mortem times, viable cells were obtained from some of the various tissues attempted: brain, spleen, liver, gut, heart, gonad, fin and gills. Of these we obtained continuously proliferating cells from fin tissues and cells have been maintained for over 5 mo. The fin cells have been authenticated by DNA barcoding as *P. flavescens,* and have been passaged over 7x to date and several flasks have been cryopreserved. Leibovitz (L-15) medium, with either 10 or 20 % fetal bovine serum (FBS), with supplemental antibiotics was successfully used for obtaining primary cultures and for further passaging into a cell line. Detailed cellular and molecular characterization of the cell line is currently in progress.

**A-2022**

TRF2 Degradation and DNA Damage Accumulation in *LMNA* Mutant Atypical Werner Syndrome Fibroblasts. B. SAHA, G. Zitnik, S. Johnson, Q. Nguyen, G. M. Martin, and J. Oshima. Department of Pathology, University of Washington, Seattle, WA 98195–7470. Email: sahab@u.washington.edu

Laminopathy is a group of genetic disorders caused by mutations in genes encoding intermediate filaments of nuclear lamina. A subset of adult-onset progeroid syndromes as described in cases of atypical Werner syndrome (AWS) patients is caused by mutations in the *LMNA* gene which encodes A type lamins. Cultured fibroblasts over expressing mutant lamin A found in AWS, undergo accelerated telomere attrition and accelerated replicative senescence. In primary fibroblasts derived from AWS patients carrying either R133L or L140R mutations, protein levels of shelterin components protecting telomeres, were significantly reduced in young passages, where cells still retained long telomeres comparable to those of young control fibroblasts. Interestingly, mRNA level of TRF2, the key component of shelterin, remained unchanged.-which suggested posttranscriptional regulation of TRF2 expression in *LMNA* mutant cells. Further examination revealed that the reduction in protein levels of shelterin subunits in *LMNA* mutant primary fibroblasts were not corrected by introducing hTERT expression but was partially restored by proteasome inhibitor treatment. This suggests that in *LMNA* mutant cells the reduction of the shelterin protein levels occurred prior to the shortening of the telomeres. In addition, *LMNA* mutant primary fibroblast exhibited increased γ-H2AX foci in non-telomeric regions of DNA. Increased γ-H2AX signals in non-telomeric DNA were also present in *LMNA* mutant hTERT cells, raising the possibility that non-telomeric DNA damage may be triggering a p53 mediated signal that leads to TRF2 degradation in *LMNA* mutants. Suboptimum shelterin-telomere interactions may thus lead to a loss of protection of telomeric ends and subsequent telomere attrition

**A-2024**

Generation of Human Induced Pluripotent Stem (iPS) Cells in Serum- and Feeder-free Defined Culture from Fetal Lung Fibroblasts and Dental Pulp Cells Derived from a Patient with Cleidocranial Dysplasia. SACHIKO YAMASAKI[1], Akira Shimamoto[2], Hidetoshi Tahara[2], and Tetsuji Okamoto[1]. [1]Department of Molecular Oral Medicine and Maxillofacial Surgery, Division of Frontier Medical Science, Graduate School of Biomedical Sciences, Hiroshima University, 2–3, Kasumi-1, Minami-ku, Hiroshima 734–8553, JAPAN and [2]Department of Cellular and Molecular Biology, Division of Integrated Medical Science, Graduate School of Biomedical Sciences, Hiroshima University, JAPAN. Email: sayamasaki@hiroshima-u.ac.jp

Human Embryonic Stem (hES) cells and human induced Pluripotent Stem (hiPS) cells are commonly maintained on

inactivated mouse embryonic fibroblast as feeder cells in medium supplemented with FBS or proprietary replacements. Use of culture media containing undefined or unknown components has limited the development of applications for pluripotent cells because of the relative lack of knowledge regarding cell responses to differentiating growth factors. Therefore we developed a serum-free medium, designated hESF9, in which human ES cells can be maintained in undifferentiated state without feeder cells. We have successfully generated hiPS cells using Yamanaka's factors (Oct3/4, Klf-4, Sox2, c-Myc) with retroviral vectors in serum- and feeder-free defined culture conditions seeded in several extracellular matrix coated dishes (type I collagen, gelatin, fibronectin, laminin). As a result we successfully generated hiPS cells using human fibroblast and dental pulp cells under the defined culture conditions, moreover the cells retained the property of self-renewal as evaluated morphologically and have an undifferentiated phenotype by virtue of the expression of the transcription factor Oct3/4, Nanog, Sox2, Esg1, Rex-1 and alkaline phosphatase. Furthermore, we found that the iPS cells generated from pulp cells from cleidocranial dysplasia (CCD) express the hES cell marker proteins, Oct3/4, Nanog, TRA-1-60, TRA-1-81 and SSEA-4. We have developed a serum-free defined medium for culturing hiPS cells, which can maintain proliferation, self-renewal and pluripotency. As this simple serum-free adherent monoculture system will allow us to elucidate the cell responses to growth factors under defined conditions, and can eliminate the risk might be brought by undefined pathogens. In addition, iPS cells derived from CCD caused by the heterozygous germ-line mutation of runt-related protein2 (RUNX2), would be beneficial to clarify the molecular mechanism involved in the disease.

**A-2026**

Direct and Indirect Effects of Interleukin-33 on Osteoclast Differentiation In Vitro. Y. MINE[1], Y. Yamaguchi[1], S. Makihira[2], H. Tanaka[1], M. K. Furue[3], and H. Nikawa[1]. [1]Department of Oral Biology and Engineering, Division of Oral Health Science, Graduate School of Biomedical Sciences, Hiroshima University, Hiroshima, JAPAN; [2]Section of Fixed Prosthodontics, Division of Oral Rehabilitation, Faculty of Dental Science, Kyushu University, Fukuoka, JAPAN; and [3]Laboratory of Cell Cultures, Department of Disease Bioresources, National Institute of Biomedical Innovation, Osaka, JAPAN. Email: yuichimine@hiroshima-u.ac.jp

Osteoclasts are multinucleated cells derived from hematopoietic stem cells. Accumulated data on the molecular mechanisms of osteoclast differentiation have demonstrated that RANKL is essential for osteoclast differentiation and maturation. It is known that one of the primary sources of RANKL is the matured osteoblast in local bone tissues and several cytokines including Interleukin (IL)-1 involved in RANKL expression in osteoblasts. IL-33 is a member of the IL-1 family cytokine, which was recently identified as the ligand for the orphan receptor ST2L. However, there is littele information available on the role of IL-33 in the differentiation of osteoclasts. Hence the aim of presented study was to examine the direct and indirect effects of IL-33 on osteoclast differentiation in vitro. Upregulated RANKL expression was observed immediately after the stimulation of MC3T3-E1 osteoblastic cells by IL-33 and the high level of expression was observed for at least 3 h. On the other hand, IL-33 are directly inhibited the RANKL-dependent expression of TRAP, CathepsinK and DC-STAMP mRNA on RAW264.7 osteoclastic cells. These results, taken together, suggested that IL-33 plays an important role in bone metabolism.

**A-2028**

Human Body-on-a-chip: A Tool to Study Health Disorders That Involve Modulations of the "Methylation Pathway". M. ARIZA-NIETO, J. M. Prot, and M. L. Shuler. Department of Biomedical Engineering, Cornell University. 305 Weill Hall, Ithaca NY 14853. Email: ma293@cornell.edu

Hypoadiponectinemia is implicated in multiple health disorders. Clinical studies suggest circulating adiponectin is negatively correlated with levels of neuropeptide Y in patients with NAFLD. Adiponectin appears to have a protective effect against diabetes vascular complications attributed in part to its ability to counteract hyperglycemia-mediated decrease in the number of Endothelial Progenitor Cells (EPC). New experimental evidence indicates that EPC together with hematopoietic progenitor cells (HPC) contribute significantly to tumor neo-angiogenesis. Furthermore, adiponectin expression appears to be dependent on activation of AMPK via the autocrine/paracrine loop under specific epigenetic patterns. Aberrant DNA methylation patterns are associated with many types of cancer. For all the reasons described above we are interested in understanding the faith of methyl donors implicated in the remethylation of homocysteine under extreme pathway overload and hepatic insulin resistance. The microenvironments known to affect epigenetic patterns and signaling between organs are being studied using DNA methylation arrays. The body-on-a-chip is a microfabricated device combined with cell cultures. Several cell culture chambers are interconnected by microfluidic channels and present the same order of magnitude as in vivo cellular tissue constructs. This microdevice prevents dilution of important secretory factors with numerous advantages like volume reduction, dynamic cell culture

and the possibility to accurately monitor the flow rate inside the microchannels. This human surrogate is being used to create a physiological microenvironment to study modulations of DNA methylation patterns and signaling between organ tissues such as GI/ liver/Brain and adipose tissue. Surrogate biomarkers (i.e., adiponectin and neuropeptide Y) are being developed and plan to be immobilized within the device. This research is funded by The Department of Biomedical Engineering, Cornell University.

**A-2030**

Manufactured Nanoparticle Toxicity in Mammalian and Fish Cell Lines: A Thorough Physico-chemical Characterization of Suspensions is Essential. J. M. NAVAS[1], M. Connolly[1], T. Lammel[1], P. Rosenkranz[1], M. Fernández[2], A. I. Barrado[2], M. D. Hernando[1], and M. L. Fernández-Cruz[1]. [1]Dpt. of Environment, INIA, Ctra. De la Coruña Km 7, E-28040 Madrid, Spain and [2]Mass Spectrometry and Geochemical Applications Unit, Chemistry Division, CIEMAT, Avda. Complutense 22, E-28040 Madrid, Spain. Email:jmnavas@inia.es

Manufactured Nanoparticles (NPs) have been shown to produce cytotoxic, genotoxic, inflammatory and oxidative stress responses in different mammalian cells in vitro. However nanoparticle toxicity studies in fish cell lines are not as well documented or explored. In a series of experiments carried out in our laboratory, the toxicity of a variety of NPs was determined in mammalian and fish cell lines. An array of cytotoxicity assays measuring different cellular damage and oxidative stress levels were applied. The main objective was to shed light on the mechanisms underlying the toxicity of NPs but this approach also allowed us to determine differences in sensitivities among cell lines and methods used. NPs exhibit unusual physico-chemical properties different from bulk material and their biological activity is dependent on many factors, such as size, shape, and surface properties. Therefore, to fully understand the observed toxic effects it is essential to conduct a thorough physico-chemical characterization of the suspensions in culture medium at different concentrations and time points reflecting exposure conditions. For metal oxide NP suspensions, such as CeO2, TiO2 or ZnO, important differences were observed between nominal and measured concentrations as determined by means of inductively coupled plasma – mass spectrometry (ICP-MS). Real concentrations are fundamental when explaining observed toxicity and, taken together with other factors such as NP size, can lead to an accurate assessment of nanoparticle toxicity towards a particular cell line. In colloidal suspensions, such as that of Au NPs or dendrimers, nominal and measured concentrations were similar. Toxicity was clearly dependent on concentration, but the presence of surface functional groups also played a key role. Important differences in sensitivities among cytotoxicity assays and cell lines were observed, with fish cell lines showing higher resistance to nanoparticle toxic effects than mammalian cell lines.

**A-2032**

3D-Fish Cell Culture: Some Do and Some Do Not. S. RAKERS, L. Luellwitz, C. Kruse, and M. Gebert. Fraunhofer Research Institution for Marine Biotechnology, Working Group Aquatic Cell Technology, Paul-Ehrlich-Str.1-3, 23562 Luebeck, GERMANY. Email: sebastian.rakers@emb.fraunhofer.de

3-dimensional fish cell culture systems are advantageous tools to study e.g. cell interactions, cell differentiation and tissue formation. However, cultivation of 3-dimensional cultures is difficult. A lot more has to be learned about the features that allow cultivation of tissue nnnnnn-like structures, after all about the interplay of different cell types. Recently, we showed that fibroblast-like cells from rainbow trout (RT) skin obviously helped to enhance survival rates of epithelial-like cells that were suspected to be terminally differentiated. We now made the observation that the long-term RT-fibroblast-like cell cultures of the skin started to build organoid-like structures spontaneously after long-time cultivation without subcultivation. No contact inhibition was observed. Compared to other cells from RT these cells showed an enormous growth after confluence. This was documented by timelapse imaging. RT-brain-derived cells or pancreas-derived cells from sturgeon didn't show this feature. The expression of the following markers in RT-skin derived organoid bodies has been analysed by immuncytochemistry: Cytokeratin 7, 14 and 18, Collagen Type I and Pax 6. Stem cell markers Sox2 and Oct 3/4 have been analysed, too. Overall we conclude that the ability of fish cells to grow in vitro in 3D strongly depends on the cell type, the environment in vitro and the factors secreted by the cells. Fibroblast-like cells of rainbow trout apparently provide a good platform for further research on e.g. wound healing experiments and mass production of fish cells.

**A-2034**

In Vitro Culture of Fibroblast-like Cells from Room Temperature Stored Goat Tissue. M. SINGH[1], X. Ma[1], and A. K. Sharma[2]. [1]Animal Science Division, Fort Valley State University, Fort Valley, GA 31030 and [2]Department of Laboratory Medicine and Pathology, Mayo Clinic, Rochester, MN 55901. Email: singhm@fvsu.edu

To test the postmortem storage limits of animal tissues, we cultured 2–3 $mm^2$ skin pieces (n=70) from ears of 3 breeds

of goats (n=7) after 0, 2, 4 and 6 d of postmortem storage at 24°C. Outgrowth of fibroblast-like cells (>10 cells) around the explants was scored after 10 d of culture. Our results show that all the explants irrespective of breed displayed outgrowth of cells on the dish containing fresh tissues. However, the number of explants exhibiting outgrowth reduced with increasing time interval. Only 53.85 % explants displayed outgrowth after 2 d of tissue storage. The number of explants displaying outgrowth was much smaller after 4 d (16.67 %) and 6 d (13.3 %) of storage. In general, the number of outgrowing cells per explant, on a given day, also decreased with increasing postmortem storage time interval. To test the differences between cell populations, obtained from postmortem fresh and stored tissues, we established secondary cultures from one of the goats exhibiting outgrowth of cells after 6 d of tissue storage. Comparison of both the cell cultures revealed similar cell morphology, growth curves, and had doubling times of 23.04 h & 22.56 h, respectively. These results suggest that live cells can be recovered from skin tissues of goats and perhaps other animals even after 6 d of their death with comparable growth profiles. These results suggest that animals dead at farms for several days can be used to recover tissues for preservation of genetic diversity and to recover lost genetics through animal cloning.

**A-2035**

Gut Bacteria in Human Atherosclerotic Tissue. A. MOROZOV[1], M. Chien[1], E. Kozarov[2], and S. Kalachikov[1]. [1]Columbia University Genome Center and Department of Chemical Engineering, 550 West 120th Street, Rm. 801, New York, NY 10027 and [2] Columbia University College of Dental Medicine, 630 West 168th Street, New York, NY 10032. Email: sk363@columbia.edu

Atherosclerosis has been long suspected to be associated with chronic bacterial infections originating from poorly maintained teeth and guts and leading to the buildup of bacteria in the blood vessels, which causes inflammation and degradation of the blood vessel walls. Possible sources of bacteria include dental diseases, chronic bronchitis, pneumonia, and Ludwig's angina. In this study, quantitative polymerase chain reaction (qPCR) with universal primers against 16S rRNA gene was used to compare the amount of bacterial DNA present in atherosclerotic tissue from diseased aorta *vs* healthy tissues from the same patients. We showed that the amount of bacteria, *Enterobacter* in particular, present in the diseased samples was statistically higher than in healthy tissues. To analyze the phylogenetic diversity of bacteria present in atheromata, nearly full-length segments of 16S rRNA genes were sequenced by the Sanger method using the metagenomics approach. Atherosclerotic samples contained more bacteria compared to healthy tissues, and among the identified bacteria were those usually found in human oral biofilms and intestines as well as environmental bacteria typically associated with soil and waste waters. The most abundant bacterial group in atheromata and, at the same time the one with the largest difference in amounts in diseased and healthy samples, was *Firmicutes* which has been shown recently to be associated with obesity in mice and humans. It is possible that the chronic inflammatory response of the vascular tissue to *Firmicutes*, especially in obese individuals, triggers the formation of atherosclerotic plaques. Thus, in this study, we found that atheromata contain higher levels of bacteria and identified a new possible connection between atherosclerosis and obesity.

**A-2036**

Demonstration of the Proliferation of Transformed Human Breast Cancer Cells in Malleable (Overhead Projector Sheets) and Inflexible (Cover–slips) Substratum-utility for Cellular Behavior and/or Curvature-based Studies. I Saranya, D. R. Naveen Kumar, and P. K. SURESH. School of Biosciences & Technology, VIT University, Vellore, Vellore Dt. Tamil Nadu, INDIA, PIN: 632014. Email: p.k.suresh@vit.ac.in

The relative ability of human breast cancer cells (MDA-MB-231 and MCF-7) to grow on different substrates as well as chemically-induced morphological changes (a proof-of-principle approach). The substrata used were coverslips with and without gelatin (C/W, C/WO), and OHP with and without gelatin (O/W, O/WO). Phase contrast microscopy-based visualization provided evidence for the adhesion of cells on these substrates post incubation for 3 h. This growth in the absence of fibroblasts/treated-surfaces seems to indicate their mesenchymal nature. Also, a cell viability assay was done to demonstrate the ability of cells to proliferate. $1 \times 10^4$ cells were seeded on all test substrates. Cells were stained, at 24 h intervals for 4 d, with 0.5 % crystal violet dye and absorbance was measured at 540 nm. Preliminary data seems to indicate that the cells grown in C/W and O/WO exhibited significantly ($p<0.05$) high growth physiognomies (N=3) in comparison with other substrata used in our study. These improved growth characteristics on OHP sheets as well as its better attachment could be attributed to be due to its textural properties. Further, the differentiation ability of cells was studied by treating them with 50 μg/ml of ascorbic acid and growth was continually monitored for 8 d. Preliminary results indicate that the MDA-MB-231 cells treated with ascorbic acid retained their spindle-shaped morphology, whereas the controls showed enlarged cells (light microscopic visualization). Also, our data seem to indicate a differential ability of MCF-7 to that of MDA-MB-231, in ascorbic acid-induced reversal to the cobble-stone morphology (N=3). This

work is the first of its kind to demonstrate growth on malleable OHP sheets. Use of this substratum, potentially allows the investigator to study physically-induced morphological changes thereby mimicking in vivo conditions involving curvature. Work is underway to characterize the texture dimensions of OHP sheet by Atomic force microscopy to provide a physical basis for the improved cell adhesion properties.