Pluripotent stem (PS) cells are cell entities, which potentially bear multi-lineage cell differentiation capacities. At the same time, these cells have self-renewing property. It should be kept in mind that this very property is a key hallmark, which distinguishes early embryonic cells with PS cells. Therefore, a primary prerequisite of the PS cells for any developmental event to occur is to quit this self-renewing state to allow cells to sense extracellular inducing signals and follow the ensuing genomic directions. Over the years, I’ve been involved in developing protocols, which allow neural induction to occur starting from mouse ES cells. I’ll be starting by mentioning about my earlier works related to mouse ES cell-work and continue to provide examples with our more recent successes using human iPS cells. In general, the mechanisms related to development are very similar between development in vivo and differentiation in a dish. Such a rule called “community effect” (coined by John Gurdon) depicts that the cell densities have large influence over cell lineage commitment. Therefore, we reckoned that regulating cell number in a dish might provide differentiation cues to PS cells. Our basic strategy to induce differentiation is to let the single cells form aggregates, which is a prerequisite to mimic development. We soon discovered that allowing cells to form free-size aggregates had negative impacts on uniformed differentiation. We first tested “hanging-drop” strategies, which allow a definite number of cells to form aggregates. Encouraged by our initial results and then with the generous help from Thermo Fisher Scientific, we’ve switched this process by using Thermo Scientific NunclonSpheria 96-well U bottom-plates. This cell-non-adherent surface allows one to better control cell aggregate sizes with enhanced practicality. Owing to this technical advantage, we can now derived human neurons at high efficiencies. We’ve also noted that the usage of NunclonSpheria lead to enhanced neurite formation of the neurons during its maturation stage. Another pitfall of human neural progenitors is that they are too tough to be triturated to single cells, a step essential to derived expandable neural stem cells. To this aspect, I’ve been mostly helped by HyQtase, a Thermo Scientific HyClone reagent which allowed me to triturate forming neural stem cells without cell viability loss. Altogether, we are now able to induce in a robust fashion neurons from different individuals and to directly compare their phenotypes. Using this improved neural induction platform, I’m taking part in an ongoing project (at Saitama Medical University) to compare different genotypes vis-à-vis their neuronal phenotypes among patients with mitochondrial respiratory chain disorders.

This said, there are still rooms for improvements for such iPSC-based strategies and most of these probably reside in how we derive iPSCs. PS cells which have not yet started their differentiation processes do not express any differentiation marker and are said to be in an “undifferentiated” state. A beauty about mouse ES cell, especially of 129-genetic background, is that the undifferentiated state maintained by LIF added to the culture medium is quasi-synonymous to its pluripotent state. The generality of this situation has been challenged and transformed with the derivation of human ES cell. It is now commonly accepted that human PS cell is akin to epiblast-stage embryo. The mouse equivalent to human PS cell is considered the Epiblast-like Stem Cell (EpiSC), which can be derived with similar conditions to human ES cell but has been reported to have imperfect self-renewing capability. The inability to fully maintain these cells (human PS cells and EpiSCs) in an undifferentiated state may also contribute to another characteristic coined “primed” by Smith and colleagues. Compared to mouse PS cells, primed PS cells are predisposed to differentiate into a restricted number of cell lineage(s). Therefore, in the strict sense of the word, human PS cells may not be completely “pluripotent”.

Towards practical usages of iPSCs for example, it is important to achieve a condition in which PS cells proliferate in a self-renewing fashion with reduced skewed differentiation propensity. In this talk, I will present our two strategies towards this goal. The first one is to keep human iPSCs in an epiblast-equivalent stage but reducing the differentiation propensity by first avoiding genomic integration of the reprogramming factors and then optimizing the culture condition to let cells open to any type of differentiation to ensue. In this newly established condition, without necessarily converting the cells into a blastocyst-stage, cells responded to various differentiation signals with high fidelity and hence, became “naïve” to differentiation cues. By applying this novel culture condition, I was able to obtain human neural cells in which it is possible to monitor and measure some basic neuronal properties such as axonal transport and/or mitochondrial energy production. The other more radical approach is to directly (or indirectly) induce human iPSCs toward an inner cell mass-like cell, or into the so-called “naïve” state. As an epigenetic barrier exists between these two stages, we are currently trying to manipulate and revert this process back. If this turns out to be successful, I would be happy to also share these preliminary results.

Next, I’d like to present an idea, which, in my opinion, would be formidable if not impossible to carry out without the help of iPSC technology. The idea is to screen human genes, which are under the influence of genomic imprinting. Genomic imprinting is a phenomenon where mono-allelic gene expression is observed in a parent-of-origin manner. A subset of the imprinted genes acquires a tissue-specific imprinted status during the course of tissue development but mechanisms underlying this epigenetic regulation were largely elusive. We have recently provided an answer to this enigma by exploiting hybrid mouse ES cell system. With this system, we revealed that the switch from bi-allelic expression to maternal mono-allelic expression of Ube3a, a known neuron-specific imprinted gene, occurs during the late neuronal development, neurate outgrowth period, and that the expression of endogenous antisense transcript of Ube3a locus is up-regulated several hundred-fold at the same period. This has also been a technical challenge for us to be able to cross-comparing between samples in biological- replicate samples. An important implication from this study is that evaluation of the “quality” of the cells derived from in vitro differentiation of ES cells needs scrutinizing epigenetic aspects such as genomic imprinting status found in tissues in vivo, in addition to the evaluation by differentiation gene markers and morphology. This experience of ours and the recently explored fact that the mouse brain has at least 1,300 genes under the influence of genomic imprinting has prompted us to revisit the mouse study but using human iPSC-derived neurons. As such parent-of-origin fixed monoallelic gene expression is vulnerable to a single mutation on a single allele, we reason that this type of genes could be good candidates for many neurological disorders. Because we can obtain genomic information relatively swiftly these days, RNA-seq analyses of the iPSC-derived neurons would in theory provide the long list of neuron-specific imprinted genes in human.

The other excellence of human iPSCs is that we can derive numerous cell lines from different individuals with minimal paperwork when comparing to human ESCs. But to fully benefit...
from this technology, I can probably not stress too much about the importance of deriving “proper” iPSCs in the first place and also to get access to a “robust” differentiation system. I finally hope these would be the two important messages I’d be sharing with you through my talk.

**Microgravity Facilitates Stem Cell Proliferation and Neural Differentiation after Cell Transplantation in Neurological Disorder Models**

**Louis Yuge, Dr.Med.Sc., Ph.D.**
Hiroshima University and Space Bio-Laboratories Co., Ltd.

Microgravity is known to control cell cycle, cell proliferation, and differentiation. A 3D-cinostat is a multi-directional gravity device for simulated microgravity. By controlling rotation of two axes, a 3D-cinostat minimizes the cumulative gravity vector in cells cultured at the center of the device and makes 10-3G average over time velocity. This is accomplished by rotation of a chamber at the centre of the device contained Thermo Scientific OptiCell Cell Culture System to disperse the gravity vector uniformly within a spherical volume, at a constant angular velocity. Our previous studies demonstrated simulated microgravity inhibited myoblasts and osteoblasts differentiation supporting data as gravitational space biology.

In our study, we developed the application of microgravity to stem cells culture using a 3D-cinostat. We reported microgravity potentiated stem cell proliferation such as human mesenchymal stem cells and mouse embryonic stem cells.

Recently, regenerative medicine with bone marrow stromal cells (BMSCs) has gained significant attention for the treatment of central nervous system diseases. Here, we investigated the activity of BMSCs under simulated microgravity conditions. Neural-induced mouse BMSCs (mBMSCs) cultured under 1G conditions exhibited neural differentiation, whereas those cultured under microgravity did not. Moreover, under microgravity conditions, mBMSCs could be cultured in an undifferentiated state. Next, we intravenously injected cells into a model of cerebral contusion and spinal cord injury. Graft mBMSCs cultured under microgravity exhibited greater survival in both neurological disorder models damaged region, and the motor function of the grafted mice improved significantly.

We demonstrated that culturing cells under microgravity enhances their survival rate by maintaining an undifferentiated state of cells, making this a potentially attractive method for culturing donor cells to be used in grafting by 3D-cinostat. This method has significant potential for regenerative medicine and development biology.

**Applications of scalable culture system for clinical-grade undifferentiated human embryonic stem cells**

**Eihachiro Kawase, Ph.D.**
Kyoto University, Institute for Frontier Medical Sciences

Although the cultures of human pluripotent stem cells (hPSCs), including embryonic stem cells and induced pluripotent stem cells in laboratory scale are relatively well developed, the cell culture systems for robust propagation of undifferentiated hPSCs and differentiated hPSC-derived cells (with clinical grade) still require considerable improvement. Identification of microenvironment for hPSCs such as media components and extracellular matrix is important issue to realize future therapeutic applications. Here, we first will introduce our GMP facility for manufacturing and banking of clinical-grade hPSCs. Then, we will discuss how to set up and optimize xeno-free cell culture conditions toward manufacturing clinical-grade hESCs. We also will present our recent data for gene targeting of hPSCs. In our studies, we used Thermo Scientific products not only for cell culture supplies but also evaluation of undifferentiated and/or differentiated state of human ESCs and human ESC-derived cells.

**Innovations in media design and cryopreservation protocols for reliable long term storage of stem cells**

**Amy Sinor-Anderson, Ph.D.**
Thermo Fisher Scientific, Laboratory Consumables Division

Since the development of media for cell culture use in the 1950s, optimization of media to enhance growth rates, decrease cell death, and increase shelf life of the complete media has been a primary focus for the cell culturist. Most media consists of a complex mixture of amino acids, vitamins, energy sources, growth factors, trace elements and additional components in a buffered salt solution. Each component adds to the complexity of the media and each has its own shelf life, sensitivity to the physical environment, and potential degradation products. Typical, mammalian cell culture formulations require supplementation of the media with a protein source, which is typically serum. Rather than using serum, that is largely undefined, stem cell researchers prefer to use a more defined media system. This defined media eliminates much of the batch-to-batch variation that is usually associated with serum because the components in the supplement are known. Through advances in media formulation, a serum replacement product offered by Thermo Scientific, provides an alternative to serum. Although ample cell growth and expansion is important for the stem cell biologist, cryopreservation of stem cells is vital for maintaining viable cell banks. Recovery of viable cells post-thaw can vary greatly depending on the cryopreservation media and protocols used. Thermo Scientific offers chemically defined cryopreservation media that promotes good recovery after thaw and viability. In this talk, we will discuss media design for optimal growth, expansion, decreased cell death and ways to promote better recovery of cells after cryopreservation.

**Characterization of stem cell pluripotency and differentiation using image cytometry**

**Jeffrey R. Haskins, Ph.D.**
Thermo Fisher Scientific, Cellomics Products

There is no doubt that stem cell research holds incredible promise in many areas of discovery research, as well as potential human therapeutic applications. Understanding the complex signaling processes, differentiation mechanisms and general physiology of stem cells is critical to their utilization, and often involves painstaking, manual experiments that provide only subjective results. Image cytometry offers a rapid, automated and quantitative approach to investigating stem cell biology at the cellular level, enabling increased productivity and the promise to accelerate this critical area of biological research. This seminar will review the advantages of image cytometry, current applications of the technology in stem cell research and demonstrate how image cytometry can provide a reliable technique for determining the pluripotency of stem cells, as well as allow rapid characterization of differentiated cells.
**Agenda**

8:30am - 8:45am  
Registration and Breakfast

8:45am - 8:55am  
Opening Remarks  
Chair: Cindy Neeley and Jeffrey R. Haskins, Ph.D., Thermo Fisher Scientific

8:55am - 9:45am  
Naive pluripotent stem cells as a prerequisite for modeling diseases  
Hidemasa Kato, M.D., Ph.D., Saitama Medical University, Research Center for Genomic Medicine

9:45am - 10:30am  
Microgravity Facilitates Stem Cell Proliferation and Neural Differentiation after Cell Transplantation in Neurological Disorder Models  
Louis Yuge, Dr.Med.Sc., Ph.D., Hiroshima University and Space Bio-Laboratories Co., Ltd.

10:30am - 11:00am  
Characterization of stem cell pluripotency and differentiation using image cytometry  
Jeffrey R. Haskins, Ph.D., Thermo Fisher Scientific, Cellomics Products

11:00am - 11:15am  
Coffee and Networking Break

11:15am - 11:55am  
Applications of scalable culture system for clinical-grade undifferentiated human embryonic stem cells  
Eihachiro Kawase, Ph.D., Kyoto University, Institute for Frontier Medical Sciences

11:55am - 12:25pm  
Innovations in media design and cryopreservation protocols for reliable long term storage of stem cells  
Amy Sinor-Anderson, Ph.D., Thermo Fisher Scientific, Laboratory Consumables Division

12:25pm - 12:30pm  
Closing Remarks