The 4th Annual "Induced Pluripotent Stem Cells Event: Production and Utility in Regenerative Medicine and Other Applications"

Tuesday, 04 June 2013
The Royal College of Pathologists, Carlton House Terrace, London, SW1Y 5AF, United Kingdom

This 4th Annual event will review the drawback and advantages of hIPSCs for diverse type of clinical applications.
This event has CPD accreditation
This event is part of the 2013 Euroscicon Stem Cell Trilogy. To find out more see www.stemcells2013.com

Meeting chair
Dr Lyn Healy, NIBSCC, South Mimms, UK

9:00 – 9:45 Registration

9:45 – 10:00 **Introduction by the Chairs**: *Dr Lyn Healy*, NIBSCC, South Mimms, UK

10:00 – 10:40 **Deriving Metabolically Active Hepatocytes from Pluripotent Stem Cells**
Dr David Hay, MRC Centre for Regenerative Medicine, University of Edinburgh, Scotland
Faithfully recapitulating human physiology "in a dish" from a renewable source remains a holy grail for medicine and pharma. Many procedures have been described that, to a limited extent, exhibit human tissue specific function in vitro. In particular, incomplete cellular differentiation and/or the loss of cell phenotype post-differentiation play a major part in this void. We have developed an interdisciplinary approach to address this problem, employing skill sets in cell biology, materials chemistry and pharmacology. Pluripotent stem cells were differentiated to hepatocytes before being re-plated onto a synthetic surface. Our approach yielded metabolically active hepatocyte populations that displayed stable function for over two weeks in vitro. Although metabolic activity was an important indication of cell utility, the accurate prediction of cellular toxicity in response to specific pharmacological compounds represented our goal. Therefore, detailed analysis of hepatocellular toxicity was performed in response to well defined compounds and compared to primary human hepatocytes. Importantly, stem cell derived hepatocytes displayed equivalence to primary human material. Moreover, we demonstrated that our approach was capable of modelling metabolic differences frequently observed in the population.

10:40 – 11:20 **Biophysical considerations of differentiation and reprogramming in embryonic stem cells**
Dr Kevin Chalut, University of Cambridge, United Kingdom

11:20 – 11:50 **Speakers' photo then mid-morning break and trade show**
Please try to visit all the exhibition stands during your day at this event. Not only do our sponsors enable Euroscicon to keep the registration fees competitive, but they are also here specifically to talk to you

11:50 – 12:30 **Surround yourself with support – showcasing services and products from Life Technologies™**
Dr Roland Leathers, Life Technologies, UK
This session will showcase the services and products that are available to you to support your stem cell research. We will also include a recent example where we have worked directly with the Parkinson's Institute to generate iPSC from patients. In collaboration we utilised several technologies including semi-conductor sequencing to characterise these patient cell lines.

12:30 – 13:10 **Stemming Visual Loss Using Pluripotent Stem Cells**
Professor Pete Coffey, Head of Ocular Biology & Therapeutics, UCL Institute of Ophthalmology, London, UK
The London Project to Cure Blindness was launched at the UCL Institute of Ophthalmology in June 2007, and aims to make the most of human embryonic stem cells to prevent blindness and restore sight in patients with Age-related Macular Degeneration (AMD) by 2013. Our goal is to replace cells essential for "seeing" lost by disease at the back of the eye. We aim to repair and regenerate the aged diseased eye using human embryonic stem cells which have been transformed into the cells affected in AMD: the support cells for the photoreceptors (retinal pigment epithelium) and the photoreceptors. The cells will be surgically implanted into a clinical population of AMD patients.

13:10 – 14:10 **Lunch and trade show**
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14:10– 14:50 **Using iPS cells to model embryo development**

Dr. Aida Rodriguez, University of Nottingham, UK

The development of new cellular reprogramming technologies has enabled the reversal of differentiation of somatic cells into undifferentiated cells. Modulation of the signalling pathways as well as selection of specific exogenous factors for iPS production can lead to the generation of cells with different pluripotent characteristics that can be used to recapitulate normal embryo development in vitro. Our laboratory is using the new understanding on metastable states of pluripotency to model the developmental transitions during embryo development and exploit this features to generate animal cells amenable able to contribute efficiently to germline chimeras.

Our studies are focused in the differentiation of pluripotent cells into germ cell precursors in different species. Elucidating the program of germ cell differentiation is of critical importance for developing safe technologies that will enable the generation of artificial gametes.

14:50 – 16:00 **Question and Answer Session**

Delegates will be asked to submit questions to a panel of experts. Questions can be submitted before the event or on the day

16: 00 - 16:30 Chairman's summing up

Dont forget to sign up to Euroscicons' e-newsletter at www.euroscicon.com/signup.htm to keep up to date with European Life Science news and events and to be notified of the follow up to this event

Event Web Site: **www.regonline.co.uk/IPS2013**

About the Chair

Dr Lyn Healy is the senior Stem Cell Biologist at the UK Stem Cell Bank, has over 20years experience in the stem cell field working primarily on human and murine haematopoietic stem cells and more recently focused on human ES cells. Lyn leads on the stem cell R&D for the bank.

About the Speakers

Dr David Hay is a Principal Investigator at the University of Edinburgh's MRC Centre for Regenerative Medicine. David has worked in the field of stem cell biology and differentiation over the last decade. David's work has highlighted the important role that cell physiology and chemical biology plays in the generation of predictive and drug inducible human hepatocytes from pluripotent stem cells. The impact of this work has led to a number of publications and regular appearances at high profile international conferences. Most recently David and his colleagues spun out a company from the University of Edinburgh, FibromEd, whose focus is to reduce the cost of human drug attrition.

Roland Leathers finished his PhD in cell and molecular biology at the University of Sheffield University, UK in 1989. Following a post-doc in Sheffield, he moved to the Ecole Polytechnique Federale de Lausanne (EPFL) in Switzerland to lead an industrial research collaboration with Hoffmann La Roche. Roland joined Life Technologies in 1993 and has since occupied various positions within the organization. He is currently Strategic Alliances Manager for the Stem Cell and Drug Discovery business unit with geographical responsibility across Europe, India and Australasia.

Ramiro Alberio is a Lecturer in Developmental Epigenetics at the University of Nottingham, UK. Research in his laboratory focuses in developing cellular reprogramming technologies, and in the study of pluripotency in different mammalian species. The laboratory uses stem cells from mouse, human and pig to determine the common pathways used by pluripotent cells. This knowledge is used to design protocols for iPS generation, and to study their differentiation potential in vivo and in vitro. He is co-founder of EvoCell Ltd., a University spin-off company that commercializes stem cell technologies developed in his laboratory.

NOTES ABOUT THIS EUROSCICON EVENT

For your convenience we would like to bring your attention to the following

- You will be issued with a FULL delegate list within 14 days of the event, which will include the email addresses of the delegates (we are sorry that there is this delay in emailing the list, but we need to make sure that it takes into account any late arrivals). You will not be included in this list if you have opted out and you can do this by logging into your registration details. This list will not be sold or ever give out to third parties. Only people attending or sponsoring the event have access to the list
- There may be an independent meeting report published within a few months of this event. If this is published we will send you an email to let you know the reference details
- Notepads and pens are available from the Euroscicon reception desk
- We cannot give out the slides from our speaker's presentations as they are deleted immediately after each event. If you require a particular set of slides please approach the speaker
- Please remember that EuroSciCon is a small independent company with no subsidies from society memberships or academic rates for venues. We try to be as reasonably priced as possible and our delegate rates are substantially lower than comparable commercial meeting organisations
- To keep updated on our events and other Life Science News, please sign up for our newsletter at www.eurosciconnews.com
- We may take pictures during the meeting. These pictures will be used to promote our events and placed on our various websites and the closed Euroscicon group on Facebook. If you do not want your photograph distributed please let one of the Euroscicon staff know.

POSTER PRESENTATIONS

HYALURONIDASE-LOADED PLGA MICROPARTICLES INCREASE MESENCHYMAL-LIKE CELLS AND DECREASE PULMONARY FIBROSIS

C.S. Bitencourt; G. M. Gelfuso; P.A.T. Pereira; P.A. Assis; C. Tefé-Silva; S.G. Ramos; E. C. Arantes; L.H. Faccioli
Departamento de Análise Clinicas Bromatológicas e Toxicológicas, Faculdade de Ciências Farmacêuticas de Ribeirão Preto – Universidade de São Paulo - Ribeirão Preto, SP, Avenida do Café, S/N, Monte Alegre, Ribeirão Preto, SP, Brazil, 14040-903, E-mail: faccioli@fcfrp.usp.br; Fone: +55 16 36024303.

Considering that bovine Hyaluronidase (HYAL) has been demonstrated to increase a heterogeneous population of mesenchymal-like cells (MSC-like) into bronchoalveolar space (BAL) and to reduce bleomycin-induced fibrosis, the objective of this work was to develop polymeric microparticles (MPs) loading HYAL to target the delivery of this enzyme to the lung, aiming an innovative strategy to treat pulmonary fibrosis. Unload MPs (Control-MPs) and HYAL-MPs were prepared by methods of emulsion/solvent evaporation and were properly characterized. Size distribution, zeta potential and morphology of MPs were adequate to intranasal (i.n). administration. Also, the release rate and encapsulation efficiency of HYAL were highly satisfactory. Subsequently, naive C57Bl6 mice were i.n. inoculated with sterile PBS, 16 U of soluble HYAL, 2 mg of HYAL-MP or Control-MP, and the cells recovered from bronchoalveolar space (BAS) 96 hours later. Total cell from the BAS were labelled with antibodies (anti-CD34, anti-CD73, anti-CD90.1, anti-SCA.1) conjugated with different fluorochromes. The mean of intensity of fluorescence (MIF) on the cell membrane were analyzed by flow cytometry. Our results demonstrate that MP-HYAL were more efficient than soluble HYAL to recruited MSC-like to the BAS. Following, mice were exposed to 4 U/kg of bleomycin intratracheally, and 7 days later treated i.n. with 16 U of soluble HYAL, 2 mg HYAL-loaded-MP or 2 mg Control-MP. BAL cells were recovered 96 h later and the total and differential cells enumerated. Next, histological analyzes, collagen and cytokines content in the lung tissue were performed. The results showed that HYAL-MP led to a reduction in TGF-β production and collagen deposition. Histopathological findings showed that the treatment with HYAL-MP was more efficient than soluble HYAL to reduce bleomycin-induced fibrosis, indicating the feasibility of HYAL-MP as an effective delivery system for the treatment of pulmonary fibrosis.

STEM CELL MARKERS IN ORAL EPITHELIAL DYSPLASIA: THEIR RELEVANCE AND DISTRIBUTION

S.M.M. Barakat and C.H. Siar

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Background: Stem cells, by their unique character of self-renewal, play a pivotal role in the maintenance of epithelial structural integrity as well as in the development of many diseases where this normal process is altered. Oral epithelial dysplasia (OED) is widely accepted as the predictor of malignant formation in oral mucosal lesions that carry this potential risk. **Aim:** To examine the epithelium in OED for the presence of epithelial stem cell markers and to speculate on their relevance. **Methods:** Formalin-fixed paraffin-embedded sections of 10 cases each of mild, moderate and severe OED were selected. Five cases of normal oral mucosa (NOM) served as control. Immunohistochemistry based on the EnvisionTM Detection System was performed to stain for four stem cell markers: integrin alpha6, neuron glial 2 (NG2), notch 1 (N1) and keratin 15 (K15). Their expression patterns were semiquantitatively scored in five hotspots/fields at x200 magnification for each constitutive epithelial layer. Level of immunoreactivity was scored as follows: 0 (negative/no staining), + (focal staining in <25% cells), ++ (significant staining in 25-50% cells) and +++ (predominant staining in >50%). Mean scores were obtained. **Results:** Integrin α6 was the most widely expressed marker, being strongest in the spinous layer but the overall distribution pattern was indistinctive between different grades of OED and control samples. NG2 was almost negative to absent in all samples, while nuclear N1 expression was most apparent in severe OED. K15 staining intensity in the basal layer tends to diminish with increasing grades of OED. **Conclusions:** The expression of these stem cell proteins seems mainly to be related to the differentiation stage/phenotype of cells in the constitutive layers of the normal and altered oral epithelium. They are not useful as molecular markers for predicting malignant transformation in OED.

This study is supported by the University Malaya Postgraduate Research Grant (PPP), Grant No: PS166/2010B.

THE NOVEL IPS CELLS GENERATED UNDER HYPOXIC CONDITIONS IN THE ABSENCE OF VIRAL INFECTION AND ONCOGENIC FACTORS AND USED FOR THE ISCHEMIC STROKE THERAPY

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Induced pluripotent stem (iPS) cells have the potential to cell therapy. However, at least two hurdles remain: integrating viral transgenes and introducing oncogenes: c-Myc and Klf4. We tested our hypothesis that iPS cells can be generated by introducing only Oct4 and Sox2 under hypoxic conditions and could use for cell therapy. We observed that the repeated transfection of two expression plasmids (Oct4 and Sox2) into mouse embryonic fibroblasts (MEF) and combined hypoxic condition resulted in novel iPS cells generation. Six hours post-transfection, MEF were subjected to hypoxic conditions for 24 h (3% O2); this procedure was repeated four times. The MEF were seeding on feeder cells on day 9; iPS cell clones were observed 12 days post-seeding and designated as iPS-OSH. The morphology, stem cell markers staining, gene expression profiles, embryonic body, teratoma and chimeric mice formation indicated that the iPS-OSH had pluripotent capability. We differentiated the iPS-OSH into neural precursor cells and used to the ischemic stroke mouse therapy. The behavior analysis showed the therapeutic group was better than the control group. We also observed that iPS-OSH-derived neural precursor cells differentiated into neuron and astrocyte in stroke brain. In conclusion, we generated a novel iPS-OSH in the absence of viral infection and oncogenic factors and could used for the stroke therapy.

TREATMENT OF CHRONIC LIVER FAILURE BY USING STEM CELLS TRANSPLANTATION.

Shagidulin M.[1,2], Onishchenko N.[1,2], Krasheninnikov M.[1], Iljinskyl.[1,2], Mogeiko N.[1], Lundup A.[2], Bashkina L.[1], Nicolskya A.[1], Volkova E.[1], Petrakow K.[1], Avramov P.[1], Perova N.[1], Sevastjanov V.[1] and Gautier S.[1,2].
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Background: Treatment of chronic liver failure (CLF) is the important problem of medicine and using of new more effective technologies for CLF-treatment is an actual aim. For this reason, there is the great interest in stem cells transplantation as a method of supporting and stimulating therapy at CLF, especially among patients standing on the waiting list for liver transplantation.
Methods: CLF was modeled on Wistar rats (n=100) by means of CCl_4 treatment within 6 weeks and death-rate was 20 %. Healthy adult Wistar rats (n=20) were also used as donors of multipotent mesenchymal stromal cells (MMSC). MMSC were obtained by standard procedure and were cultivated during 10 days. Then MMSC were placed on biodegradable matrixes «Sphero®GEL» and were applied in the form of biounits. It was carried out 5 experimental groups (gr.). In 7 days after CCl_4 treatment MMSC as biounits were transplanted (Tx) into damaged liver in doses 2.5×10^6 cells (gr.1) and 5×10^6 cells (gr.2). MMSC as a suspension were introduced by i/v on the 3rd day in dose $2,5\times10^6$ cells (gr.3) and on the 3rd and the 10th days in total dose 5×10^6 cells (gr.4). In gr.5 (control) saline solution was injected into damaged liver. Dynamics of liver failure reduction; of liver and biounits morphology were investigated within 90 days after cell therapy.
Results: CLF was characterized by increasing of ALT, AST, ALP, GGT, bilirubin indices and depression (decreasing) of synthetic (albumin) liver function. In liver it was determined vacuolization and necrosis of hepatocytes, progressive or total fatty and hydropic dystrophy of hepatocytes. In gr.1 and 2 the all biochemical indices returned to normal levels on the 5-7th days after Tx of biounits. Normalization of biochemical indices was observed both in gr.3 and especially in gr.4 on the 12-14th days after MMSC application. In the control group: ALT returned to normal level on the 18th day, AST returned to norm on the 90th day, ALP did not returned to norm during all experimental time. It was ascertained that in 28 experimental days the CT area in liver was increased in gr.5, but it was resolved in gr.1, 4 and especially gr.2. In 90 days the processes of liver fibrosis became more serve in gr.5, but in gr.1-4 (especially in gr.2 and gr.4) the resolving liver fibrosis took plase. Besides, in 90 days there were in liver viable hepatocytes single hepatocytes with hydropic dystrophy and double-nuclear hepatocytes in 1-4 gr. In gr.2 marked angiogenesis and bile ducts epithelium proliferation were detected.
Conclusion: It was shown that Tx or i/v application of MMSC affected as an adaptogen, mobilizing reparative reserves in damaged liver. MMSC therapy of CLF can reduce liver failure: accelerates normalization of some liver functions, accelerates resorption of fibrosis and connective tissue in liver. The proposed method is effective for the correction and treatment of CLF and can be used in clinical practice.

Keywords: iPSC, hepatocyte, liver, CYP p450, translation, supply chain, delivery, GMP, manufacturing,drug, pluripotent stem cell, hepatocyte, pancreas, liver; beta-cell, Stable Karyoytpe, High-throughput efficiency, Embryonic Stem cells, Induced pluripotency, Nanog, neural stem cell, glioblastoma, DNA methylation, reprogramming, iPS cells, reprogramming, Pluripotentcy, STEMCCA, ESGRO 2i, Pluripotency; reprogramming; chromatin signatures; DNA replication timing; histone acetyltransferase p30, hESC, Drug Metabolism, Hepatocyte, Liver, reprogramming, characterisation, Parkinsons, culture