



# PhD Project Proposals

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## Contents

<b>Dr Sajjad Ahmad</b> (p3)	Signalling mechanisms for limbal stem cells and corneal epithelial maintenance Regression of corneal blood vessels in limbal stem cell deficiency The construction of an in vitro corneal bio-construct
<b>Dr Carrie Ambler</b> (p4)	Notch signaling in human skin diseases
<b>Dr Akis Karakesisoglou</b> (p5)	Cytoskeletal and nuclear crosstalk in ageing
<b>Prof Majlinda Lako</b> (p7)	Understanding the role of TGFb1 and TGFb3 on differentiation of human embryonic stem cells (ESC) towards haematopoietic lineages
<b>Prof Hanns Lochmuller</b> (p9)	Modelling dystrophic cardiomyopathy with iPSC Establishing a conditional immortalization system to be used in patient-derived myoblasts for therapeutic evaluation studies.
<b>Dr Annette Meeson</b> (p10)	Human cardiac stem cells, can they contribute to cardiac regeneration?
<b>Dr Julia Reichelt</b> (p11)	Forcing keratinocyte stem cells out of their niche
<b>Prof James Shaw</b> (p12)	Generation of new insulin-secreting beta cells from human adult pancreatic stem cells
<b>Prof Maya Sieber-Blum</b> (p13)	Differentiation of hEPI-NCSC into dopaminergic neurons and application in an animal model of Parkinson's disease
<b>Prof Colin Jahoda &amp; Dr Arto Maatta</b> (p14)	Characterisation of a skin stem cell niche
<b>Prof Todd Marder, Dr Andy Whiting, Prof Stefan Przyborski, Dr Paul Hunt, Dr Chris Redfern</b> (p15)	Synthesis and applications of retinoids as triggers of stem cell differentiation
<b>Prof Nick Reynolds &amp; Prof Colin Jahoda</b> (p18)	Role of calcium and NFAT signaling in regulating human epidermal stem cell homeostatis
<b>Prof Stefan Przyborski &amp; Prof Neil Cameron</b> (p20)	Smart scaffolds for triggered release of bioactive molecules during 3D stem cell culture
<b>Dr Julia Reichelt &amp; Prof Maya Sieber-Blum</b> (p21)	Regulation of stem cells in the epidermis

## **Dr Sajjad Ahmad**

### **1. Signalling mechanisms for limbal stem cells and corneal epithelial maintenance**

The cornea is the clear front of the eye and its clarity is vital for normal visual functioning. The cornea is covered by an epithelium which is renewed by stem cells located at the periphery of the cornea, in a region known as the limbus. Little is known about the signalling mechanisms maintaining limbal stem cells. Gene microarray studies will be used to identify and investigate such mechanisms. By understanding these mechanisms, new treatments can be developed for patients blinded by a deficiency of limbal stem cells.

### **2. Regression of corneal blood vessels in limbal stem cell deficiency**

The cornea is the clear front of the eye and its clarity is vital for normal visual functioning. The cornea is covered by an epithelium which is renewed by stem cells located at the periphery of the cornea, in a region known as the limbus. A deficiency of these so-called limbal stem cells results in the painful and blinding disease of limbal stem cell deficiency. One of the characteristics of this disease is the presence of new blood vessels on a normally avascular cornea. The use of anti-angiogenic agents to eliminate these corneal blood vessels will be investigated using a mouse model of limbal stem cell deficiency. These studies will eventually result in the setting up of multi-centre trials in patients with limbal stem cell deficiency.

### **3. The construction of an in vitro corneal bio-construct**

The cornea is the clear front of the eye. There are approximately 6 million people blinded by corneal disease worldwide. The conventional management of corneal scarring is by the transplantation of cadaveric cornea. There is however a shortage of donor corneas. In this project, the construction of an in vitro corneal bio-construct will be investigated using stem cells and tissue engineering techniques. The cornea is composed of three main layers: an epithelium, a stroma and an endothelium. Construction of these three layers separately in the first instance will be investigated, followed by a combination of all three layers. This will be used to develop a corneal bio-construct which can be used for corneal transplantation in the future.

## **Dr Carrie Ambler**

### **Notch signaling in human skin diseases**

In humans, hair follicle clumping or “tufting” occurs in a skin inflammatory disease called tufted hair folliculitis. Tufted hair folliculitis is most often classified as a sub-category of the human cutaneous inflammatory condition keratosis folliculitis decalvans. Clinical studies strongly suggest that human tufted hair folliculitis is caused by a chronic *Staphylococcus Aureus* infection. Histological data show dermal infiltration of neutrophils and lymphocytes localised adjacent to the upper portion of the hair follicle, and it has been hypothesised that following immunocyte infiltration, fibrosis around the upper follicle results in contraction causing the tufted appearance. Tufted hair folliculitis is also classified as a scarring alopecia, or permanent loss of hair, but a recent study suggests initial tufting arises from hair follicle re-spacing as the lower portion of the hair follicles are retained.

In our studies we found that forced Notch activity in epidermal progenitor cells led to abnormal clumping of hair follicles with the areas in between largely devoid of hair giving the skin a “tufted” appearance. Occasionally, we found hair follicles shared a common infundibulum or upper portion of the follicle, yet the hair follicle bulge and bulb remained distinct. Hair follicle tufting was irreversible as mice retained their tufted phenotype following a 4-month chase period after drug-induced epidermal Notch activity. We have correlative evidence that Notch-induced hair tufting is linked to inflammation. A great number of dermal lymphocytes were found in transgenic mice with evident hair “tufting”. Additionally, injecting mice with the anti-inflammatory dexamethasone while activating the Notch pathway reduced the number of hair tufts. As far as we can discern from the published literature, hair follicle re-spacing leading to “tufting” is unique to our mouse model of skin inflammation.

One goal is to determine whether Notch-induced hair tufting in mice mimics this human inflammatory disease. In collaboration with Dr. Andrew Messenger, a consultant dermatologist at the Royal Hallamshire Hospital in Sheffield, we hope to look at whether samples from human patients with tufted hair folliculitis have evidence of dysregulated Notch signaling.

## Dr Iakowos Karakesisoglou

### Cytoskeletal and nuclear crosstalk in ageing

Ageing is accompanied at the cellular level by nuclear malfunctioning and profound cytoskeletal reorganization. The molecular changes that lead to such alterations, however, are poorly understood. Moreover, it is unknown whether these two topologically distinct phenotypes are linked with each other and whether they contribute to the cessation of cell proliferation in ageing tissues.

A novel group of nuclear envelope proteins, the Nesprins and the Sun-proteins may hold the key in the biology of ageing. In fact, Nesprins are involved in premature ageing diseases such as Hutchinson Gilford progeria. Preliminary data indicate also that Nesprin-loss induces cellular senescence and controls several aspects of nuclear architecture including centrosomal positioning. Furthermore, Nesprin/Sun complexes connect the genetic material with all major cytoskeletal elements, providing a pathway of communication between the nuclear and cytoplasmic compartments.

The aim of the project is to elucidate, how changes in the Nesprin/Sun signaling cascades during ageing may affect skin homeostasis *in vivo* and *in vitro*. Specifically, we will document the phenotype of loss of function Nesprin/Sun protein epithelial cell mutants and examine the roles in cell fate determination, cell proliferation and their ability to form a tissue in organotypic *in vitro* cell cultures. Moreover, we aim to characterize Nesprin/Sun functions in the skin tissue of young and aged rodents. These studies will be supplemented by *in vitro* model studies, utilizing cell lines where cellular senescence has been introduced. Finally, by validating and examining a number of yeast-two hybrid Nesprin-associated proteins implicated in cytoskeleton organization, gene regulation, including telomerase expression we envision unraveling novel molecular pathways that underpin normal ageing.

My group pioneers this area of research and possesses all the required reagents (Nesprin/Sun antibodies, siRNAs, cDNAs, dominant negative interference approaches), cell culture and mouse models for the successful execution of this proposal. The student will be skilled in cell biology, biochemistry, imaging techniques and gain expertise in an emerging field in the biology of ageing.

#### Peer-reviewed Publications:

\*\*Kandert S., Lüke Y., Kleinhenz T., Neumann S., Lu W., Jaeger V.M., Munck M., Wehnert M., Müller C.R., Zhou Z., Noegel A.A., Dabauvalle M.C., Karakesisoglou I. (2007) Nesprin-2 giant safeguards nuclear envelope architecture in LMNA S143F progeria cells. *Hum. Mol. Genet.* 16: 2944-2959.

Peche V., Shekar S., Leichter M., Korte H., Schröder R., Schleicher M., Holak T.A., Clemen C.S., Ramanath-Y.B., Pfitzer G., Karakesisoglou I., Noegel A.A. (2007) CAP2, cyclase-associated protein 2, is a dual compartment protein. *Cell Mol. Life Sci.* 64: 2702-2715.

\*\*Padmakumar V.C., Libotte T., Lu W., Zaim H., Abraham S., Noegel A.A., Gotzmann J., Foisner R., and Karakesisoglou I. (2005). The inner nuclear membrane protein Sun1 mediates the anchorage of Nesprin-2 to the nuclear envelope. *J. Cell Sci.* 118, 3419-3430.

\*\*Libotte T., Zaim H., Abraham S., Padmakumar V.C., Schneider M., Lu W., Munck M., Hutchison C., Wehnert M., Fahrenkrog B., Sauder U., Aebi U., Noegel A.A., and Karakesisoglou I. (2005). Lamin A/C Dependent Localization of Nesprin-2, a Giant Scaffold at the Nuclear Envelope. *Mol. Biol. Cell* 16, 3411-3424.

**Dr Majlinda Lako**

## **Understanding the role of TGF $\beta$ 1 and TGF $\beta$ 3 on differentiation of human embryonic stem cells (ESC) towards haematopoietic lineages**

The transforming growth factor beta (TGF $\beta$ ) superfamily contains highly pleiotropic members that encompass diverse functions during embryogenesis and adult tissue homeostasis. Mammalian members of the TGF $\beta$  superfamily include TGF $\beta$ 1, TGF $\beta$ 2, TGF $\beta$ 3, Activins, Inhibins, BMPs (bone morphogenetic proteins), GDFs (growth differentiation factors), GDNFs (glial derived neurotrophic factors), Nodal, Lefty etc. There are two branches of TGF $\beta$  signalling: the TGF $\beta$ /Nodal/Activin branch and the BMP signalling branch. The TGF $\beta$ /Nodal/Activin branch of TGF $\beta$  signalling involves the activation of intracellular Smad2/3, which becomes phosphorylated and complexes with co-Smad4 before being translocated to the nucleus. The other branch, the canonical BMP signalling pathway is initiated by the binding of BMP to heterodimers of BMPRI $\alpha$  and BMPRII. This leads to activation of Smad1/5/8, which forms a heteromeric complex with Smad4 prior to translocation to the nucleus. Suppressors of cytokine signalling (SOCS) and inhibitory Smads such as Smad7 provide negative feedback regulatory mechanisms.

In vertebrates, strong evidence exists to support a crucial role for both branches of this family in fate specification within the primitive streak, mesoderm development, emergence of haematopoietic cells from mesoderm and proliferation of haematopoietic cells. Our own work has shown that two members of this family, TGF $\beta$ 1 and TGF $\beta$ 3 act as positive enhancers of human ESC haematopoietic differentiation (Ledran et al. 2008). Together these data suggest an important role for several members of the TGF $\beta$  family during early haematopoietic development. Notwithstanding this, the precise action and target cell types in which TGF $\beta$ s act during early human embryonic development is not known. This is the key thrust of this proposal outlined herein which will aim to focus on three important lines of investigation as follows:

- Define the expression of TGF $\beta$  superfamily members (in particular, the TGF $\beta$ /Nodal/Activin branch) during differentiation of human ESC to mesoderm, specification of haemangioblast and further differentiation to haematopoietic and lineages. We will make use of our efficient differentiation protocol already reported in Ledran et al. 2008, *MIXL1*-GFP marked human ESC line obtained on collaboration basis from Profs. Elfanty and Stanley (Monash, Australia) and the ability to FACS purify selected cell populations of interest on the basis of cell surface markers such as PDGFR $\alpha$ +MIXL1+ (mesoderm), CD31+KDR+ (haemangioblast) and CD34+CD45+ (haematopoietic progenitors).
- Define the role of members of the TGF $\beta$ /Nodal/Activin branch during each stage of haematopoietic differentiation of human ESC (specification of mesoderm, emergence of haemangioblast and commitment to haematopoietic cells) using both inhibition and overexpression based studies (reported in Ledran et al. 2008) and the cell culture tools described in the above section. Once the cell type (mesoderm vs haemangioblast or

hematopoietic precursor) is identified, microarray expression profiling will be carried out to investigate molecular targets of this pathway.

- Investigate the role of TGF $\beta$ /Nodal/Activin branch members on the engraftment ability of human ESC derived haematopoietic progenitors in NOD/LtSz-*Scid IL2R $\gamma$ <sup>null</sup>* immunocompromised recipients. Our own work has shown that co-culture of human ESC with the stromal cell line derived from the aorta region of aorta-gonad-mesonephros region results in an efficient engraftment of hESC derived blood cells and this co-culture is associated with high levels of *TGF $\beta$ 1* and *TGF $\beta$ 3* mRNA levels. In view of this, we will stimulate and inhibit TGF $\beta$ 1 and TGF $\beta$ 3 pathway and assess the impacts on engraftment in the blood system of these animal models.

Reference:

Ledran MH, Krassowska A, Armstrong L, Dimmick I, Renstrom J, Lang R, Yung S, Santibanez-Coref M, Dzierzak E, Stojkovic M, Oosterndorp RAJ, Forrester L and **Lako M** (2008) Efficient haematopoietic differentiation of human embryonic stem cell on stromal cells derived from hematopoietic niches. *Cell Stem Cells* 3:85-98.

## **Professor Hanns Lochmüller**

### **1. Modelling Dystrophic Cardiomyopathy with iPSCs**

A substantial proportion of Duchenne Muscular Dystrophy (DMD) patients develop dilated cardiomyopathy which becomes a life-limiting condition. However, very little is known regarding the phenotype of dystrophic cardiomyocytes, despite much work being done on skeletal muscle. Recent work has shown that cells with a pluripotent, embryonic stem (ES) cell phenotype can be derived from fibroblasts (induced pluripotent cells or iPSCs). This provides a unique opportunity to develop a model system for dystrophic cardiomyocytes, since ES cells can readily be differentiated into cardiomyocytes.

The student will derive fibroblast cultures from DMD patient skin biopsies, as well as control fibroblasts, and use them to derive iPSC lines which will be differentiated into cardiomyocytes. These will be used to examine the impact of dystrophin deficiency on cell viability, calcium handling properties, susceptibility to mechanical and osmotic stress and differentiation potential. Where dystrophic cardiomyocytes display aberrant properties, for example showing an increased propensity towards a fibrotic fate under stressful conditions, these properties will form the basis of novel intervention strategies designed to ameliorate the cardiac phenotype in DMD.

### **2. Establishing a conditional immortalization system to be used in patient -derived myoblasts for therapeutic evaluation studies**

Studies in musculoskeletal disorders are mainly carried out in mouse models and established mouse or rat muscle cell lines. When analysing new drugs or treatment strategies it would be beneficial to be able to bring this testing to patient level, since the effects seen in mice cannot be translated directly into humans. Myoblasts, the skeletal muscle stem cells hold a great therapeutic research potential. However, the use of these cells is hampered by poor growth and early senescence of these cells in culture. This project is therefore to establish a system to conditionally immortalize these human myoblasts.

The student will derive myoblast cultures from DMD and LGMD2B patients, and healthy control subjects. These cells will then be transduced with a construct containing a tetracycline-inducible promoter system using lentiviral vectors. Different cell cycle regulators such as Cdk's or SV40 large T-antigen in combination with telomerase will be tested as potential new constructs. This is necessary since telomerase alone fails to induce immortalization of primary myoblasts. Once immortalized cell lines have been established these will be used for novel therapeutic research including ongoing drug trials currently undertaken in mice and human fibroblast cultures.

**Dr Annette Meeson**

**Human cardiac stem cells, can they contribute to cardiac regeneration?**

In preliminary studies we have observed that the developing fetal human heart is enriched for stem cells that have a side population phenotype. These cells are defined by their expression of Abcg2 which confers on them the ability to efflux Hoechst 33342 dye. In studies on mouse cardiac side population (CSP) cells it has been shown that these cells can differentiate towards a more mature cardiac lineage in vivo and in vitro suggesting that these CSP could be useful in the clinical setting as a cardiac stem cell based therapy. However, little is known about human cardiac stem cells. We have now optimised protocols for isolating CSP cells from human fetal heart tissue. The aims of this proposed study would be to determine the ability of these human cells to proliferate and differentiate into functional cardiomyocytes. This will involve differentiating these cells in vitro and in vivo and determining if they exhibit the morphological, proteomic, transcriptional and functional characteristics of adult cardiomyocytes.

## **Dr Julia Reichelt**

### **Forcing keratinocyte stem cells out of their niche**

Cells and tissues are able to respond to physical force. Mechanical stress can cause diverse biological responses like cell proliferation or differentiation and is involved in developmental processes and regeneration.

In the epidermis keratinocytes are exposed to a wide range of loads. Multipotent keratinocyte stem cells (KSC) residing in the hair follicle bulge are unique being additionally subject to tensile forces from the arrector pili muscle (APM) which inserts at the bulge region. The connection between KSC niche and this muscle suggests a specific mechanical regulation of KSC that will be investigated in this PhD project.

Wnt and  $\beta$ -catenin are known to be essential for hair follicle development, cycling and de novo hair follicle growth. The project will focus on studying the involvement of Wnt pathways in mechanical signalling. KSC spheres, the generation of which has been recently established in this lab and which mimic the bulge environment, will be biaxially stretched to different parameters using the Flexcell system. The Flexcell apparatus is a computer-controlled vacuum system allowing stretching cells grown on flexible supports. The cellular response will be studied using Western blotting, immunofluorescence analysis, TCF reporter plasmid (TOPflash) assays, qPCR and live cell imaging. Results obtained from cell culture experiments will be transferred to wound healing studies including pharmacological stimulators/inhibitors of the APM in transgenic KSC reporter mice (Krt15CrePR;R26R) which allow tracking of KSC and their progeny (HO licence in place). Migration, proliferation and differentiation of labelled KSC will be analysed on skin sections and in whole mount preparations, using BrdU labelling and keratinocyte differentiation markers respectively. Clonal analysis of keratinocytes isolated from wounds will reveal whether APM stimulation may result in depletion of KSC from their niche.

The ultimate goal of this research is to establish whether epidermal KSC can be stimulated to increase skin wound healing through manipulation of the APM via pharmacological agents.

**Dr James Shaw**

## **Generation of new insulin-secreting beta cells from human adult pancreatic stem cells**

Successful transplantation of isolated islets or whole pancreas has allowed a limited number of individuals with diabetes to stop injecting insulin. Widespread clinical implementation is prevented by insufficient suitable donor tissue. Generation of new insulin secreting beta cells from adult pancreatic stem cells may enable insulin independence in many individuals from a single donor.

Human islet-like structures has been attained from putative adult pancreatic stem cells *in vitro* following culture condition manipulation and growth factor treatment. Insulin biosynthesis and glucose-stimulated secretion remains inadequate for clinical efficacy.

The role of sequential induction and repression of specific transcription factors in the development of  $\beta$ -cells and ultimately mature islets from pancreatic progenitor cells is becoming increasingly apparent. Our group and others have demonstrated induction of insulin gene expression and insulin secretion in rodent ductal and acinar cells following growth factor treatment and transcription factor transfection. Evidence that glucagon-like peptide (GLP-1) may play a pivotal role in inducing end-differentiation in islet precursors is rapidly accumulating. GLP-1 transfection has not previously been evaluated and there is an overall dearth of data relating to human tissue.

The hypothesis to be tested in the current studentship is that:

' $\beta$ -cells or mature islets with sufficient glucose-regulated insulin secretion for clinical efficacy can be generated from pancreatic stem cells residing within adult pancreas'. Specific aims are:

To isolate, establish in primary culture and characterise stem cells from human cadaveric donor pancreas

To evaluate the potential for endocrine following growth and differentiation factor treatment (including HGF; betacellulin; GLP-1)

To evaluate the potential for endocrine neogenesis following transcription / differentiation factor gene transfer (including PDX-1; neurogenin-3; ISL-1; GLP-1)

Characterisation before and after transdifferentiation will comprise RT-PCR, Western blot and immunocytochemistry for a range of stem cell and endocrine markers; determination of insulin storage and glucose-stimulated insulin secretion by ELISA; and *in vivo* validation in diabetic rodents.

1. This work will increase basic understanding of how insulin-producing cells are made which may ultimately lead to new treatments to prevent onset and progression of Type 1 and Type 2 diabetes.
2. Generation of new insulin-producing cells from the expanded stem cells residing within adult pancreas would allow many people with diabetes to benefit from a single donor.
3. Successful expansion from a limited amount of starting material may enable transplants to be performed with a small portion of pancreas from a living donor or the individual with diabetes.

## **Professor Maya Sieber-Blum**

### **Differentiation of hEPI-NCSC into dopaminergic neurons and application in an animal model of Parkinson's disease**

This project focuses on directed differentiation of human epidermal neural crest stem cells (hEPI-NCSC) into dopaminergic neurons and their application in an animal model of Parkinson's disease. hEPI-NCSC represent a novel type of neural crest-derived multipotent adult stem cell. The neural crest is a transient embryonic tissue that generates a diverse array of cell types and tissues in the adult organism, including catecholaminergic neurons. Catecholaminergic neurons synthesize the neurotransmitters dopamine and norepinephrine. hEPI-NCSC are thus attractive candidate stem cells for dopaminergic differentiation. Preliminary data indicate that directed differentiation in culture is feasible and robust. Main techniques involved in this project include microdissection of human skin, cell culture, immunocytochemistry, real-time polymerase chain reaction, fluorescence microscopy, and confocal microscopy. Depending on his/her progress, the student may also learn animal surgery and small animal imaging. For further information contact Prof Maya Sieber-Blum by email at [maya.sieber-blum@ncl.ac.uk](mailto:maya.sieber-blum@ncl.ac.uk).

## **Dr Arto Maatta and Professor Colin Jahoda**

### **Characterisation of a skin stem cell niche**

The hair follicle harbours at least three different stem cell populations: epidermal stem cells, melanocyte stem cells and mesenchymal stem cells. The epidermal stem cell niche in the hair follicle bulge is supported by a specialised extracellular matrix, the glassy membrane. We have carried out an initial proteomic characterisation of the glassy membrane in rat whisker follicles. Stem cell niche proteome includes proteins involved in matrix assembly and stabilisation. One such identified protein is Inter-alpha-trypsin Inhibitor, (I $\alpha$ I) which forms covalent complexes with proteoglycan bikunin. The first aim of this project is to characterise localisation and expression of bikunin and associated I $\alpha$ I proteins in hair follicle stem cell niche during hair cycling and wound healing. Cell cultures enriched for epidermal or hair follicle –derived mesenchymal stem cells will be utilised to study of bikunin and I $\alpha$ I protein function by RNAi. The second aim is to conduct a large scale purification and proteomic analysis of the hair follicle basement membrane to further characterise the matrix components that contribute to the stem cell niche. The described project is designed to increase our understanding on how extracellular matrix modulates stem cell niches in skin.

**Prof Todd B. Marder, Dr Andy Whiting, Dr Stefan Przyborski (NESCI), Dr Paul Hunt (Department of Biological and Biomedical Sciences, Durham University), Dr Chris Redfern (Northern Institute for Cancer Research, Newcastle University)**

#### **Synthesis and Applications of Retinoids as Triggers of Stem Cell Differentiation (4)**

Retinoids are a class of natural and synthetic compounds which are structurally or functionally related to All-Trans Retinoic Acid (ATRA). ATRA, the oxidative metabolite of Vitamin A, has extensive biological activity, being critical to normal embryonic development and, for example, to the development of the nervous system. ATRA is also widely used to trigger stem cell differentiation. However, there are serious problems regarding reproducibility and homogeneity of cell types when this reagent is employed, especially due to the photoinstability of the compound under ordinary laboratory lighting, giving rise to numerous isomers which bind and activate one or more of 6 retinoic acid/retinoid X receptors (RAR  $\alpha, \beta, \gamma$ ; RXR  $\alpha, \beta, \gamma$ ). In contrast, we have begun to develop synthetic retinoids which are photostable and which are both more active and more selective than ATRA in triggering stem cell differentiation, giving rise to homogeneous cell cultures. For example, one such compound provides neurons whereas an isomer of it provides epithelial plaques.

In addition, these two compounds perform completely differently when applied to chick limbs during early development using polymer bead technologies. The first predominantly causes the expected limb bud duplications with more activity than ATRA, whereas the latter compound results predominantly in nasal collapse with little evidence for an effect on the limb buds in which the bead is implanted. ATRA causes both outcomes at high frequency.

Finally, given the use of ATRA in treatment of neuroblastoma, and its inherent toxicity, we are investigating the activity (especially with regard to apoptosis) of our synthetic retinoids in neuroblastoma cells.

With northeast regional SME partner High Force Research Ltd. we have developed methodology to scale up our synthesis of these two compounds which are being commercialised by Reinnervate Ltd., a Durham University spin-out specializing in enabling stem cell technologies to whom our patent has been assigned. We work closely with both SMEs, and both are currently funding our work via EPSRC and BBSRC Industrial CASE Studentships. Thus, we have formed a very successful and productive collaborative team including 2 synthetic chemists, 2 biologists, 1 cancer researcher, and 2 local SMEs. We have filed one patent and published 3 papers to date with several more manuscripts being prepared for publication and a second patent being considered. All PhD students have been jointly supervised by at least 2 team members, and have included full time chemists, full time biologists and those who have carried out research in both chemical and biological aspects of the project.

In order to expand our efforts, both in terms of commercially valuable small molecule reagents for stem cell differentiation, as well as fundamental scientific studies of their mechanism of action and development of well-defined structure-activity relationships, we require additional Ph.D. students working with our team in the following areas:

Synthetic chemistry: we require additional synthetic chemists to prepare and characterise additional known as well as novel retinoids, especially those which are expected to bind/activate individual receptors with high selectivity. This is also required in order to probe SAR. The student will utilise novel synthetic routes including proprietary catalytic technologies developed to convert inexpensive hydrocarbons into such high value added retinoids. The student will also have the benefit of our close connections with High Force Research Ltd. through which practical aspects of industrial scale up can be explored.

Metabolism studies: At present, we do not have any information on the metabolism of our synthetic retinoids. There is scarce data available on this aspect of the work although many such compounds are known. However, there is no doubt that both activity and toxicity are related to the rate of metabolism of the compounds, and both are important issues, the latter being especially important to potential clinical applications ranging from neuroblastoma chemotherapy to regenerative medicine. Our proteomics data suggests the possibility that the high activity of some of our compounds may be related to their lack of metabolic pathways.

Binding assays to the 6 retinoid receptors: Critical to our SAR studies is the establishment of both binding constants and agonist/antagonist behaviour of each of the synthetic retinoids. There are scattered reports in the literature using different tests of activity and binding, but no consistent set of data is available at present.

Investigation into the biological activity of small molecules on stem cell differentiation. A major component of this initiative is to establish the biological mode of action of small molecules created by the chemistry team, with specific interest in the ability to direct the differentiation of stem cells in a robust and reproducible manner. For example, a specific project would be based around a cohort of compounds, to investigate the molecular mechanisms by which they influence cell behaviour and the characterization of the differentiation process. Information from such experiments will provide feedback to the chemists to enhance compound design and development. The student(s) would join a postdoc to receive daily supervision and training in molecular and cellular biology, cell culture and imaging will be provided. Thorough biological evaluation of compounds is currently a bottleneck in our research programme, given the complexity of the differentiation model, time taken to investigate mechanism of action, characterisation of the differentiation response and lack of an efficient assay for rapid parallel screening of compounds.

Biological screening of effects in vivo. An initial in vivo assay which allows sensitive quantitative comparison of the effects of different compounds is the implantation of polymer beads into chick limb buds, causing alterations to the number and type of digits formed. In addition to their effects on limbs, retinoids are well known to affect a number of other aspects of embryonic development such as establishment of the dorsal ventral axis of the eye, cardiac outflow tract septation, facial process outgrowth and neural tube closure. Further investigation of the biological properties of synthetic compounds is likely to identify further examples of specificity in biological effects. This work may also link to the metabolism studies described under project 2. This would involve working alongside an existing ASGBI PhD student supervised by PNH and SAP on a project to investigate the regulatory targets of the two compounds mentioned in the introduction in chick embryos.

It is expected that aspects (2) and (3) could be performed by a single PhD student, who would be expected to assist in the development of new assays for rapid screening. Aspects (4) and (5) are significant projects in their own right and each requires a separate student.

Thus, our proposal is for a total of 4 PhD students with current priority order being:

PhD student 1: Aspect 4

PhD Student 2: Aspects 2&3

PhD Student 3: Aspect 5

PhD Student 4: Aspect 1

## Prof Nick Reynolds and Prof Colin Jahoda

### Role of calcium and NFAT signalling in regulating human epidermal stem cell homeostasis

Background: Quiescent stem cells reside in the basal layer of the epidermis and “bulge region” of the hair follicle and through proliferation and differentiation maintain skin tissue homeostasis and function<sup>1</sup>. Stem cells are also activated during wound healing and dysfunctional control of stem cell proliferation may also contribute to hyperproliferative diseases such as psoriasis. The signals regulating epidermal stem cell quiescence and proliferation are however poorly understood. Calcium ions play an important role in regulating keratinocyte proliferation/differentiation/apoptosis and we have recently shown functional activation of Ca<sup>2+</sup>/NFAT signalling in epidermal keratinocytes with abnormal activation in psoriatic epidermis<sup>1</sup>. Moreover, recent evidence indicates that one NFAT isoform NFATc1 regulates epidermal stem cell quiescence and down-regulation of NFATc1 increases stem cell proliferation<sup>2</sup> and that spontaneous Ca<sup>2+</sup>/NFAT signalling may be important in maintaining “stemness” and that Ca<sup>2+</sup>/NFAT signalling is down-regulated as stem cells differentiate<sup>3</sup>.

Hypothesis: Ca<sup>2+</sup>/NFAT signalling plays a key role in regulating epidermal stem cell homeostasis, proliferation and differentiation

Methods: Culture of normal human keratinocytes on feeder layers in defined medium and isolation of “holoclones”, “meroclones” and “paraclones” for further analysis using live cell confocal imaging of Ca<sup>2+</sup> flux using Ca<sup>2+</sup> indicator dyes and cells retrovirally transduced with GFP-NFATc1. Spontaneous and agonist-induced Ca<sup>2+</sup>/NFAT signalling will be analysed and correlated with proliferation, cell cycle progression and differentiation of colonies. Molecular tools are available to block Ca<sup>2+</sup> entry and NFAT signalling and the effect of these on stem cell homeostasis will be assessed.

Outcome: increased understanding of signalling mechanisms regulating epidermal stem cell homeostasis.

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## Dr Stefan A. Przyborski and Prof. Neil R. Cameron

### Smart Scaffolds for Triggered Release of Bioactive Molecules during 3D Stem Cell Culture

The *in vitro* culture of differentiated cells in three dimensions (3D) is used increasingly to provide cell cultures that are more representative of an *in vivo* environment, leading to better tissue and disease models. The investigators have developed a robust 3D cell culture platform, consisting of highly porous emulsion templated polystyrene-based materials, that has been shown to produce highly functional 3D cultures of a wide variety of cell types (including hepatocytic, osteoblastic, neuronal and mesenchymal stem cells) [Bokhari et al., 2007a; Bokhari et al., 2007b; Bokhari et al., 2007c; Carnachan et al., 2006; Hayman et al., 2005; Hayman et al., 2004]. To date, such scaffolds have consisted of unfunctionalised polymers and have provided a controlled physical, rather than chemical, environment for cell growth. In this project, we will extend this work to the development of surface-functionalised scaffolds that, ultimately, will be able to release bioactive molecules, in a triggered fashion, to control stem cell differentiation and/or fate.

The investigators have recently developed plasmachemical treatment methodology that introduces reactive sites onto the surface of the scaffolds. These can be used to anchor bioactive molecules for subsequent release during 3D culture. Molecules of particular interest are the retinoid derivatives developed by Przyborski and colleagues, which cause efficient and controlled differentiation of human pluripotent stem cells [Christie et al., 2008]. These will be attached to functionalised scaffolds via degradable linkers, such as thioesters, causing their release into the medium during culture. A second generation of scaffolds will be designed to release retinoids in response to the application of (low intensity) light, by means of a photocleavable linker (e.g. an *o*-nitrophenyl ester). Thus, the retinoid, or other bioactive factor, can be released into the medium at selected timepoints during the culture experiment. Combinations of both types of linker will allow the release of mixtures of factors, one at a constant and slow rate during the experiment and the other in a burst, on application of light. The effect of not only the bioactive species, but also the point at which it is released, on stem cell differentiation can be investigated in this manner, as well as the interplay between different factors.

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## **Dr Julia Reichelt and Professor Maya Sieber-Blum**

### **Regulation of stem cells in the epidermis**

The epidermis is exposed to a wide range of physical force and keratinocytes are known to react to mechanical stress with increased proliferation causing epidermal thickening at sites of strain. Multipotent keratinocyte (KSC) and epidermal neural crest stem cells (EPI-NCSC) reside in the bulge of the hair follicle. The bulge is a stem cell niche within the epidermis of the hair follicle. KSC and EPI-NCSC are unique insofar as they are subjected to tensile forces from contractions of the arrector pili muscle (APM) which inserts at the bulge region. The physical proximity between these two types of stem cell with each other and with the APM suggests that KSC and EPI-NCSC communicate with each other and may be influenced or controlled by APM contraction.

Physical stimuli are sensed and integrated by distinct cell types, including stem cells, and depending on the context initiate diverse biological responses including cell proliferation, differentiation, developmental processes and regeneration. This PhD project will focus on studying the impact of APM contractility on the fate of bulge stem cells. To this end inducible transgenic reporter mice for KSC (Krt15CrePR;R26R) and EPI-NCSC (Wnt1-cre::R26R) (allowing tracking of both types of stem cell and their progeny) will be used to follow interaction between stem cell type and their response to mechanical stimulation by the APM. The major neurotransmitter at the APM is noradrenaline. Therefore the APM will be pharmacologically modulated in these mice using agents including sympathomimetic and sympatholytic drugs respectively. Migration, proliferation and differentiation of KSC and EPI-NCSC will be analysed on skin sections and in whole mount preparations, using BrdU labelling and keratinocyte and melanocyte differentiation markers. Moreover norepinephrine transporter knockout mice (NETKO), which are deficient in norepinephrine reuptake from the synaptic cleft, with hair loss and wound healing impairment, will be used to study the fate of epidermal stem cells residing in the bulge under normal conditions and in wound healing situations. In addition, the response of co-cultured (GFP-labelled) KSC and EPI-NCSC to mechanical stretch will be studied using the Flexcell system.

Results from these studies will provide important biological insight into the regulation of stem cells in the epidermis.