THE ROYAL COLLEGE OF SURGEONS OF EDINBURGH

Research Report 2012–2014

FROM HERE, HEALTH

Contents

Foreword from the President

THE Royal College of Surgeons of Edinburgh is embarking on a new period of development of the College buildings. This has proved to be an exciting and challenging project.

The benefits of this project will be obvious once the building work is completed. However, the challenges we face in fundraising have stimulated the College to consider continuing fundraising activities but this time perhaps directed more towards research.

You will be aware that the College's research priorities are translational research, clinical research, cancer research and education and training, together with patient safety through harnessing data on surgical outcomes.

Within these broad areas, the Research Allocation Committee sees exciting possibilities for future fundraising efforts.

The Research Strategy Committee continues to contribute to the success of the College in the area of research by providing direction to the Grant Allocation Committee.

I am very grateful to both these committees for the work they do in ensuring that the funds that we have raised are allocated appropriately and produce good-quality research. The ambition for the Research Strategy Committee is to work with the Heritage Management Board to engage in fundraising for further research which will probably be in collaboration with the university institutions across Scotland and the rest of the United Kingdom.

In this way, we can help to support exciting new initiatives for the benefit of surgical patients.

Ian Ritchie, President, RCSEd

Introduction

THE range of 'surgical research' is considerable from basic science in *Drosophila* or zebrafish to the health economics of daybed surgery. Given this spectrum, it is sometimes difficult to determine what is worthy of funding and what is not. Equally, it can be difficult for applicants to discern what might be attractive to a funding committee.

The first issue is that 'quality matters' and that if a project is not based on sound methodology with results that can be subjected to peer review and put into the public domain, it will be unlikely to be funded. Secondly, due to its craft-based nature, surgery (and thereby surgical research) should mostly have a strong clinical component (or at least an element that may be translated into the clinical environment). Thirdly, if undertaken by a trainee, the research should be of benefit to career development and should take place in an environment that can support research of the highest calibre. Finally, the financial support requested must be justified in sufficient detail to allow the committee to make a judgement on value for money.

It is a pleasure to report on the high quality of projects that have been submitted to and funded by the College. The College is keen to fund not only career academics who eventually seek a university position, but also those wishing to be exposed to the research environment and who will eventually practise within the NHS. Both destinations are valued equally. To this end, we have re-instituted the joint MRC/RCSEd three-year PhD Fellowship scheme, continue to offer one-year Fellowships (e.g. Maurice Wohl Fellowship and Robertson Trust Fellowship) and have increased the upper limit to the funding offered for individual small project grants.

It is always a pleasure to nurture academically minded medical students and the summer bursary scheme continues to grow and flourish. The Syme Medal has been the subject of intense competition with the quality of applicants outstanding. The King James IV Professorship continues to be our most prestigious award and marks a lifetime of academic achievement. The Ophthalmology sub-committee continues to provide a high level of grant support and fund projects of great distinction.

The activity of the Committee and the number of awards made is clearly dependent on resources, and we are extremely grateful to the donors of research funds who help sustain such vital activities. It is hoped that current fundraising efforts within the College will add to the resource available and embellish further our extensive programme of research funding.

Professor Kenneth Fearon
Chairman, the Royal College of Surgeons of Edinburgh, Research Allocation Committee

Research Allocation Committee

CHAIRMAN

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Professor of Surgical Oncology, University of Edinburgh, Department of Clinical and Surgical Sciences, Royal Infirmary of Edinburgh

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University of Aberdeen

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Professor C M Steel, Retired

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Mr C Widgerowitz
Senior Lecturer in Orthopaedics, Division of Surgery and Oncology, Ninewells Hospital and Medical School, Dundee

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Ms A Rooney
Chief Executive, Royal College of Surgeons of Edinburgh

Ophthalmology Sub-Committee

CHAIRMAN

Professor K C H Fearon
Professor of Surgical Oncology, University of Edinburgh, Department of Clinical and Surgical Sciences, Royal Infirmary of Edinburgh

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Consultant Ophthalmic Surgeon,
Moorfields Eye Hospital, London,

Professor Andrew Dick
Department of Ophthalmology, Cellular and Molecular Medicine, University of Bristol, School of Medical Sciences, Bristol

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Professor B Dhillon
Consultant Ophthalmic Surgeon,
Princess Alexandra Eye Pavilion, Edinburgh

Mr G Dutton
Consultant Ophthalmologist

Mr R Hellewell
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Honorary Treasurer,
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Ms A Rooney
Chief Executive
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Donors to the RCSEd Research Fellowships and Grants 2012–2014

Mr Iain Fraser

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Cutner Memorial Bequest Fund

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Robertson Trust

Royal Blind

Medical Research Council

Arthritis Research UK

Lorna Smith Charitable Trust Research Fellowship

The College and the Research Allocation Committee gratefully acknowledge the donations from numerous Fellows of the College in the UK and overseas.

The Robertson Trust Research Fellowship

AWARDED TO:

Miss Jennifer Jones – Clinical Research Fellow/ RMO Spire Murrayfield
Edinburgh Urological Cancer Group, University of Edinburgh

"Evaluation of molecular markers for predicting risk of progression in high-grade non-muscle invasive bladder cancer (HGNMIBC)" (£50,000)

Predicting the risk of disease progression in high-grade non-muscle invasive bladder cancer (HGNMIBC) continues to be a challenge for urologists. Approximately one-third of patients never experience disease recurrence, one-third of patients will require cystectomy for control of local disease, and one-third of patients ultimately die of metastatic disease.

Evidence suggests that patients who undergo cystectomy for progressive HGNMIBC experience inferior outcomes when compared with patients who have a primary tumour that invades muscle at the diagnosis. HGNMIBC continues to be managed by organ-sparing transurethral resection of bladder tumours. It is not possible to predict which patients with HGNMIBC may benefit from early cystectomy based on established clinical and pathological prognostic variables alone.

This study will examine the genetic, proteomic and morphological features of HGNMIBC, and attempt to identify molecular markers that predict a poor outcome of HGNMIBC. The aim is to combine models of risk stratification with prognostic molecular markers to produce a robust 'signature' of aggressive HGNMIBC. This strategy could be applied to the selection of patients for clinical trials of early cystectomy *versus* standard bladder-conservation therapies, with the intention of improving outcomes from HGNMIBC.

Travelling Fellowship Awards

CUTNER TRAVELLING FELLOWSHIP IN ORTHOPAEDICS

Mr Kedar Deogaonkar – Trauma and Orthopaedics, Sheffield
"Clinical Fellowship – Spinal Surgery" – USA **(£750)**

Mr Nicholas Ohly – Trauma and Orthopaedics, Edinburgh
"Clinical and Academic Fellowship in Adult Reconstruction, Hip and Knee Arthroplasty" – Canada **(£750)**

Mr Samuel Molyneux – Trauma and Orthopaedics Surgery, Edinburgh
"Clinical and Research Fellowship in Adult Orthopaedic Trauma" – Canada **(£750)**

Mr James Turner – Spinal SpR, Hampshire
"Voluntary Fellowship" – Malawi **(£750)**

Mr Graham Dall – ST7 Ortho SE Scotland, Edinburgh
"Clinical Fellowship – Mercury Institute for Foot and Ankle Reconstruction, Baltimore, USA" **(£500)**

Mr James Beazley – SpR Trauma and Orthopaedics, Coventry
"Orthopaedic deformity correction Furlong Fellowship, Beit CURE International Hospital, Blantyre, Malawi" **(£2,000)**

Mr Sammy Hanna – Specialty Registrar in Trauma & Orthopaedics, Hertfordsire
"Adult Reconstruction Fellowship (Hip and Knee), London Health Sciences Centre, University of Western Ontario, London, Canada" **(£500)**

THE JOHN STEYN TRAVELLING FELLOWSHIP IN UROLOGY

Mr Aidan Noon – NIHR Clinical Lecturer in Urology, Sheffield
"Society of Urologic Oncology" – Toronto **(£900)**

King James IV Professorship Awards

Professor David Thomas – Professor/Honorary Consultant in Oral and Maxillofacial Surgery, Cardiff
"Studies on cellular and molecular control of impaired wound healing: development of novel nanomedicine approaches to 'old wounds'"

Professor Hamish Simpson – Professor of Orthopaedics and Trauma, Department of Orthopaedic Surgery, University of Edinburgh
"Understanding and preventing fracture non-unions"

Professor Crispian Scully – Emeritus Professor, University College London
"Emerging infections in health, disease and oral healthcare"

Professor Peter Anderson – Consultant Cranio-maxillofacial Surgeon, Australia
"Developing adjuvant therapy for craniosynostosis"

Small Research Grants

Mr Siong-Seng Liau – University of Cambridge
"Dissecting the roles of partner and localiser of BRCA2 (PALB2) in progression of pancreatic adenocarcinoma through conditional mouse models"
(£10,000)

Mr Michael Hughes – Royal Infirmary of Edinburgh
"Paracetamol metabolism after liver resection"
(£9,331)

Dr Shirjel Alam – University of Edinburgh
"Assessment of inflammatory-cell injury in chronic renal allograft rejection by USPIO MRI"
(£9,775)

Mr Neil Johns – University of Edinburgh
"Clinical classification of cancer cachexia phenotypic correlates in human skeletal muscle"
(£9,500)

Mr Alistair Brydone – University of Glasgow
"Osseointegration of oxygen plasma-treated 3D nanopatterned PEEK polymer implants in a rabbit intramedullary model"
(£9,512)

Mr Rhys Clement – University of Edinburgh
"Elucidating the causes of chondrocyte death in septic arthritis caused by *Staphylococcus aureus*"
(£10,142)

Mr Michael Hughes – Royal Infirmary of Edinburgh
"Energy expenditure and regeneration after liver resection"
(£9,050)

Dr Campbell Roxburgh – University of Glasgow
"Establishing crosstalk between local and systemic inflammatory reactions in colorectal cancer"
(£9,940)

Bursaries for Undergraduate Elective or Vacation Awards

Miss Jenny Ferguson – Unit of Experimental Therapeutics, University of Glasgow
"Role of Src kinase family members in the transition from primary to metastatic colorectal cancer"
(£900)

Mr Sayinthen Vivekanantham – Department of Neurosurgery, Charing Cross Hospital, Imperial College London
"Brain-section analysis of PD, MSA and PSP patients to correlate histopathological findings with recorded axial motor deficit"
(£1,500)

Miss Hui Qi Crystalline Lim – School of Life Sciences, University of Glasgow
"Cadaveric study on Becker flaps and lateral arm flaps"
(£800)

Miss Clare Connelly – Department of Surgery, University of Edinburgh
"Cell viability of matrix-loaded chondrocytes for cartilage transplantation subjected to different irrigation fluids and hydrostatic pressures"
(£1,500)

Miss Chiara Ventre – Centre for Inflammation Research, Queen's Medical Research Institute, Edinburgh
"Study into lymphocyte-mediated neutrophil activation and recruitment in hepatic ischemia-reperfusion injury"
(£296)

Miss Nina Goergen – General Surgery, Aberdeen Royal Infirmary
"Demographic characteristics, geographical distribution, and temporal changes of pre-hospital trauma deaths in Scotland"
(£1,050)

Syme Medal Awards

Mr Richard Skipworth – Specialist Registrar and Honorary Clinical Lecturer in Upper GI Surgery, Edinburgh
"A study of skeletal-muscle wasting in cancer cachexia"

Mr Alexander Aarvold – Specialty Trainee Registrar, Trauma and Orthopaedic Surgery, Wessex Deanery
"Bone tissue engineering: experimental strategies and clinical application"

Mr Daniel Ezra – Locum Consultant Ophthalmologist, Moorfields Eye Hospital and UCL Institute of Ophthalmology, London
"Tensional homeostasis in acquired upper-lid hyperelasticity: tissue changes and clinical implications"

Mr Thomas Madura – Specialist Registrar in Plastic Surgery, North Western Rotation
"Novel experimental strategies to improve functional outcome after peripheral nerve injury"

Mr Ernest Azzopardi – Clinical Academic Lecturer, Cardiff University
"Polymer therapeutics: a novel paradigm in the treatment of infection (doctoral study)"

Miss Karen Eley – Craniofacial Fellow, Oxford University Hospitals NHS Trust
"Imaging the craniofacial skeleton: is MRI a viable alternative to ionising radiation?" and "Improving the peri-operative management of patients undergoing free tissue transfer for malignancy in the head and neck"

Mr Nathan Stephen – Surgical Registrar, Higher Surgical Training Rotation, West of Scotland
"Discover of molecular biomarkers and physiological assessment of skeletal muscle in cancer cachexia"

Mr Ricky Bhogal – Specialist Registrar, in Hepato-Pancreatico-Biliary Surgery/Liver Transplantation, Birmingham
Role of oxidative stress and CD154-mediated reactive oxygen species in regulation of the death of hepatocytes during hypoxia and hypoxia-reoxygenation"

Grant / Fellowship Reports

Grant/Fellowship Reports

Novel electrochemical patterning of titanium alloy to control osteogenesis at the bone–implant interface

Sarah Johnson-Lynn
Institute of Cellular Medicine, Newcastle University, Newcastle, UK

Arthritis Research UK, Orthopaedic Clinical Research Fellowship
1 April 2009 to 31 March 2012

LAY SUMMARY

We investigated a method of treating titanium alloy implants, which are used in orthopaedic surgery for joint replacement. Experiments revealed that this method of surface treatment changed surface roughness and chemistry of surfaces. We investigated how these surfaces influenced the growth and behaviour of bone cells on them.

Cell-culture experiments showed that treatment of the metal significantly altered the size and appearance of the cells grown on their surfaces and changed focal adhesion complexes (i.e. areas of the cell responsible for adhesion to surfaces.

We found that a protein involved in communication between cells, cadherin-11, was significantly different on these surfaces. Significant differences were found between surfaces in terms of the activity of Ras homolog gene family, member A (RhoA), a molecule involved in cell signalling. These results highlighted that the electrochemical processing of titanium alloy can affect the biology of bone cells.

The amount of bone in contact with the implants (which were placed in rat tibias) was measured using CT, mechanical testing and microscopy. We noted that the significant changes to the amount of bone in contact with the implant surface were dependent upon surface treatment. These results suggest that the electrochemical processing approach described could be used to influence the way in which bone anchors titanium implants, thereby increasing their longevity.

GRANT REPORT

(A) CLINICAL AND SCIENTIFIC SIGNIFICANCE OF ADVANCES MADE

The main achievements of the project were to fully characterise the morphological and chemical properties of surfaces created on titanium alloy by a novel process of electrochemical etching. This was achieved using light microscopy, atomic force microscopy, scanning electron microscopy and X-ray photoelectron spectroscopy. Significant differences in mean roughness were produced by treating surfaces using the processing parameters of 3 V and 5 V compared with 9 V. Surface chemistry was also altered, with reduced levels of vanadium and aluminium found in the surface alloy layers of the 9 V-treated surfaces.

The early response of bone precursor cells to these surfaces was investigated using rat primary calvarial osteoblasts and human mesenchymal cells in short-term cultures. Significant differences were observed in cell polarity and cell area between the rat and human cells cultured for 24 h on surfaces treated at 3 V and 5 V and those treated at 9 V. Significantly greater mean focal adhesion area and mean number of focal adhesions per unit cell area were also observed between 3 V- and 5 V-treated surfaces compared with 9 V-treated surfaces. The extent to which these changes were due to surface morphology (rather than surface chemistry) was assessed using polycaprolactone reproductions of experimental surfaces and repeating cell-

Novel electrochemical patterning of titanium alloy to control osteogenesis at the bone–implant interface

culture experiments. The pattern of changes in cell morphology on polycaprolactone surfaces was similar to that of processed titanium alloy, suggesting that surface topography had a greater effect on cell morphology.

Longer-term cell-culture experiments were employed to investigate the function and differentiation of cells towards a phenotype of mature osteoblasts using an alkaline phosphatase activity assay after 14 days of culture. These studies revealed no significant changes in the activity of alkaline phosphatase between cells cultured on the different surfaces, but a trend towards greater activity on 9 V-treated surfaces was observed. After 28 days in culture, cells were stained for mineralised bone-like deposits: no significant differences were seen in the total area of bone nodules found on the experimental surfaces.

To begin to assess how the surfaces created in this study influenced the biology of bone cells, studies focused on cell–cell communication (cadherins) and small guanosine-5′-triphosphate (GTP)ases, which are known to reflect the structure and organisation of the cytoskeleton. We used immunofluorescence to detect and localise cadherin-11, activity assays for the small GTPases Ras-related C3 botulinum toxin substrate (Rac)1 and RhoA, as well as inhibition assays for Rac1 and Rho-associated kinase (ROCK). On 9 V-treated surfaces, significantly increased cell-surface staining for cadherin-11 was seen, suggesting greater cell–cell communication via adherens junctions. Significantly greater RhoA activity was observed at 24 h on 9 V-treated surfaces compared with those on 5 V-treated surfaces, but no trend was seen in the results for Rac1. Upon inhibition of Rac1 and ROCK for 24 h, the decrease in focal adhesion staining with anti-vinculin was reduced by a significantly greater amount on 3 V- and 5 V-treated surfaces than on 9 V-treated surfaces. This finding suggested that their action in controlling cell morphology is more important in cells that have less established adhesion whereas, on surfaces in which cell spreading is pronounced, Rac1 and ROCK play a less significant part.

Finally, titanium-implant materials treated to recreate the experimental surfaces were implanted in rat tibias. After 21 days, tibias were retrieved and the bone–implant interface investigated using mechanical push-out testing, histomorphometry and micro-CT. Histomorphometric analyses revealed a trend towards a greater area of mineralised bone matrix in contact with the implant surface for 9 V-treated surfaces and the same trend was observed (and statistical significance reached) upon analyses of micro-CT images. The variability in the dataset for mechanical testing was very large, so no significant differences were seen. However, a trend was seen for greater load-to-failure of the bone–implant interface for 3 V-treated surfaces compared with 9 V-treated surfaces. The 3 V-treated surfaces were known to be significantly rougher and these observations were not accompanied by a corresponding trend of increased bone–implant contact. Hence, we suggest that the mechanical-testing results were due to ‘mechanical interlocking’ of the bone trabecule with the rougher surface.

Established treatment for end-stage osteoarthritis is joint replacement. Replacement of the hip and knee are the most widely undertaken and successful procedures, and improve the lives of many patients significantly. With the changing demographics of the UK population, greater numbers of older people require joint-replacement surgery and many are keen to

Novel electrochemical patterning of titanium alloy to control osteogenesis at the bone–implant interface

remain active in their later years. This situation not only challenges the provision of primary joint replacement but also increases the numbers of patients requiring revision joint replacements due to failure of the original procedure. One of the more frequent causes of the failure of joint replacement is aseptic loosening, in which components become dislodged from their fixation after a period of implantation. Factors affecting this scenario include: the quality of the initial fixation; motion at the bone–implant interface; stress-shielding of the surrounding bone. These factors can be addressed by changes to the surface of the implants, thereby increasing the abundance and organisation of bone fixation.

The method of electrochemical modification investigated in this project can: be scaled up to industrial production; be used to treat any shape of implant; produce different surfaces on different parts of an implant; have potential cost and safety implications for production because it does not require lithographic masks for patterning and does not require hazardous chemicals such as hydrofluoric acid (which is commonly used for similar applications). This project also adds to the knowledge of how implant surfaces affect the bone–implant interface by their effect on cell–cell signalling and intracellular signalling cascades. This project has, therefore, identified an approach to improve biological interactions with titanium-implant materials leading to bone formation, and investigated the aspects of cell biology that underpin these events. The next challenge is to translate these findings into orthopaedic biomaterials.

(B) PROBLEMS ENCOUNTERED AND STEPS TAKEN TO OVERCOME THEM

In the early stages of the study, we encountered difficulties with the reproducibility of the electrochemical modification of the surfaces. The methodology was novel and needed to be modified from a previous version for it to be suitable to treat samples for cell-culture experiments. After several iterations, the problem was resolved with greater quality control of the electrolyte solution and some alterations to the electrochemical cell. It was hoped that some significant changes in expression of osteogenic genes would be seen upon analyses of real-time quantitative polymerase chain reaction of the cells after seven days in culture. However, the variability of these data was large, despite extensive repetitions of the experiment, so meaningful conclusions could not be drawn. We were aware that the reporting of mechanical push-out testing in the literature concluded that this method of measurement often yielded high variability in the results. Due to the ethical, time and cost constraints inherent in a doctoral project, we could not test sufficient numbers of implanted rat tibias for the observed trend in this experiment to reach statistical significance.

(C) COLLABORATIONS ESTABLISHED

- Centre for Electrochemistry and Advanced Materials, Newcastle University
- Dental Materials Laboratory, Centre for Oral Health Research, Newcastle University
- EPSRC, Leeds University
- Bruker microCT (Kontich, Belgium)
- Centre for Comparative Biology, Newcastle University

Novel electrochemical patterning of titanium alloy to control osteogenesis at the bone–implant interface

(D) PUBLICATIONS AND PRESENTATIONS (INCLUDE ANY PRIZES AWARDED), HIGHER DEGREE AND FURTHER FUNDING OBTAINED AS A RESULT OF PRESENT AWARD

Publication

Birch MA, Johnson-Lynn S, Nouraei S, Wu Q-B, Ngalim S, Lu W-J, Watchorn C, Yang T-Y, McCaskie AW and Roy S. Effect of electrochemical structuring of Ti6Al4V on osteoblast behaviour *in vitro*. *Biomedical Mater* 2012; 7: 1–14.

Presentations

1. Johnson-Lynn S, McCaskie AW, Birch MA. Novel electrochemical patterning of titanium alloy to control osteogenesis at the bone–implant interface. Poster presented at: Clinical Academic Training Annual Meeting, Newcastle University, May 2010; Institute of Cellular Medicine Research Day, June 2010; British Orthopaedic Research Society meeting, 12 July 2010.

2. Johnson-Lynn SE, McCaskie AW, Birch MA. Electrochemical treatment of titanium influences osteoblast activity. Poster presentation, British Orthopaedic Research Society meeting, June 2011.

3. Johnson-Lynn SE, Roy S, McCaskie AW, Birch MA. Novel electrochemical patterning of titanium controls osteoblast morphology and behaviour. Poster presentation, British Orthopaedic Association meeting, September 2011.

4. Johnson-Lynn S, Roy S, McCaskie AW, Birch MA. Novel electrochemical patterning of titanium alloy to control osteogenesis at the bone–implant interface. Oral presentation, Arthritis Research UK Fellows meeting, March 2012.

5. Johnson-Lynn SE, Roy S, McCaskie AW, Birch MA. Osseointegration *in vitro* and *in vivo* of electrochemically modified titanium surfaces. Oral presentation British Orthopaedic Research Society annual meeting, September 2012.

Doctoral thesis complete and ready for submission.

(E) ACKNOWLEDGEMENTS

- Dr MA Birch, Institute of Cellular Medicine, Newcastle University
- Professor AW McCaskie, Institute of Cellular Medicine, Newcastle University
- Professor S Roy, Centre for Electrochemistry and Advanced Materials, Newcastle University
- Dr M German, Centre for Oral Health Research, Newcastle University
- Dr J Varia, Centre for Electrochemistry and Advanced Materials, Newcastle University
- Dr J Portoles, Centre for Electrochemistry and Advanced Materials, Newcastle University
- Arthritis Research UK
- RCSEd

Grant/Fellowship Reports

Employing a high-throughput, quantifiable proteomic approach to the development of a panel of markers predictive of outcome from sunitinib treatment in patients with metastatic renal cell cancer

Alexander Laird
Edinburgh Urological Cancer Group,
Institute of Genetic and Molecular Medicine, University of Edinburgh, Edinburgh, UK
The Robertson Trust Research Training Fellowship
1 August 2011 to 1 August 2012

LAY SUMMARY

Approximately 50% of patients with renal cell cancer (RCC) eventually develop metastatic disease. Sunitinib is the most commonly used targeted therapy for metastatic RCC but all patients eventually develop resistance. Tests to predict patient response to treatment have lagged behind those for other tumour types. This may be (at least in part) a result of the multiple subpopulations of cells present within the tumour. The variance in subpopulations ('heterogeneity'), and the effect of treatment on heterogeneity, has not been established at the protein level.

By analysing the expression of 55 key proteins in the pathogenesis of RCC in multiple areas of 23 treatment-naïve and 27 sunitinib-treated nephrectomy samples, we confirmed intra-tumoural heterogeneity in RCC. Furthermore, we have shown that there is increased intra-tumoural heterogeneity after sunitinib treatment. Despite these data, there are significant differences in the median level of protein expression of 30 proteins between treated and untreated patients. Six of the most important markers were confirmed to show the same trends in a small cohort of matched pre- and post-sunitinib-treated RCC samples. Three of these proteins, if combined with clinical parameters, were shown to be predictive of the response to sunitinib treatment.

This work (which reflects most of the work in the literature) studied changes in primary RCC to understand the pathology of RCC and the response to treatment. However, the aim of sunitinib treatment is to reduce metastatic burden, so differences in heterogeneity and protein expression between primary RCC and metastatic RCC were assessed. We confirmed that heterogeneity remains in metastatic disease, but that there are significant differences in the expression of key proteins between primary RCC and metastatic RCC tissues. Further study of these differences may provide understanding of the response to treatment and could highlight the need for biopsy of metastases in routine clinical care.

Employing a high-throughput, quantifiable proteomic approach to the development of a panel of markers predictive of outcome from sunitinib treatment in patients with metastatic renal cell cancer

GRANT REPORT

(A) CLINICAL AND SCIENTIFIC SIGNIFICANCE OF ADVANCES MADE

1. HETEROGENEITY AND DIFFERENTIAL PROTEIN EXPRESSION PRE- AND POST-SUNITINIB THERAPY

Fresh frozen tissue was obtained from 23 sunitinib-naïve and 27 sunitinib-treated nephrectomy samples. From each tumour, frozen sections were made and up to five protein lysates obtained from each spatially and morphologically diverse region of each tumour. Reverse-phase protein arrays (RPPAs) were set up to assess the levels of 55 proteins relevant to RCC pathogenesis and sunitinib activity.

Significant intra-tumoural variance in protein expression that was exacerbated by sunitinib was identified. Forty of the 55 proteins examined had greater intra-tumoural variance in the sunitinib-treated group. Thirty proteins were expressed differentially between sunitinib-naïve and sunitinib-treated patients. Consistent changes after sunitinib treatment were: increased expression of proteins associated with angiogenesis; increase in expression of members of the mechanistic target of rapamycin (mTOR) pathway; decrease in mismatch repair genes.

The trends for expression of key proteins (B-cell lymphoma 2, MutL homolog 1, carbonic anhydrase IX, mTOR, N-cadherin, epithelial cell adhesion molecule (EpCAM)) were validated using automated quantitative analysis (AQUA) of immunofluorescence. N-cadherin, EpCAM, and mTOR were selected as predictive features in a model of overall survival that performed well in sunitinib-treated (training data) and sunitinib-naïve (test) groups.

This work showed that there is significant protein heterogeneity in metastatic RCC that is exacerbated by sunitinib therapy. Despite this finding, potential novel mechanisms of resistance and predictive biomarkers have been identified. These require further development and validation for translation to the clinic.

2. HETEROGENEITY AND DIFFERENTIAL PROTEIN EXPRESSION BETWEEN PRIMARY AND METASTATIC RCC TISSUE

Much of the literature on pathway analyses in the development of molecular therapies for RCC focuses on the primary tumour. The first part of this work also assessed differences in protein expression in the primary tumours of patients with metastatic RCC after sunitinib treatment. However, the aim of such treatments is to reduce metastatic burden, so understanding the differences between the primary tumour and metastatic tumours at a molecular level is crucial.

Fresh frozen tissue was obtained from 20 primary RCCs and six sites of metastases. Protein lysates were extracted from up to four areas of each specimen. RPPA was used to show significant intra-tumoural variance in primary and metastatic RCC. Despite this finding, there was significant differential protein expression of cluster of differentiation (CD)10, epithelial membrane antigen (EMA), BCL2 and Aurora A between primary tumours and metastases. These findings are being validated on tissue microarrays of 163 primary tumours and 69 metastases in triplicate using AQUA. Because of the small cohort used for this RPPA analysis, other selected key proteins that did not reach statistical significance in RPPA studies were also studied in the AQUA cohort. This study showed significantly greater expression of Ki67, p53, vascular endothelial growth factor receptor (VEGFR)1 and the transcription factors

Employing a high-throughput, quantifiable proteomic approach to the development of a panel of markers predictive of outcome from sunitinib treatment in patients with metastatic renal cell cancer

◁ SLUG and SNAIL in metastases compared with primary tumours.

These results confirmed significant differences in selected protein expression between primary RCCs and their metastases. They also highlight the need for further study of RCC metastases to understand the response to treatment and to overcome resistance. Establishing the differences between primary tumours and their metastases may highlight the need for routine biopsy of metastases.

(B) PROBLEMS ENCOUNTERED AND STEPS TAKEN TO OVERCOME THEM

The project proposal had detailed analyses of the *in vitro* studies of the effect of sunitinib on RCC cell lines to identify lines with varying sensitivity to drugs. We undertook assessment of the proteins and pathways involved in the response of RCC cell lines to sunitinib treatment over time and at different concentrations before proceeding to tissue analyses. However, it was felt that the tissue analyses would provide the most representative changes related to sunitinib treatment in RCC. Nonetheless, this cell-line work is underway to allow more in-depth analyses of pathway activation in development of the sunitinib response and correlation of results using clinical tissue. It is anticipated that the results from the tissue analyses will be confirmed and that the cell-line models will be used to manipulate pathways and dissect the mechanisms of resistance.

(C) COLLABORATIONS ESTABLISHED

- Professor Tom Powles, principal investigator: SuMR project (REC: 10/S1402/33). Access to matched sunitinib-naïve and treated metastatic RCC tissue.
- Dr Ian Overton, MRC Human Genetics Unit. Bioinformatics support

(D) PUBLICATIONS AND PRESENTATIONS (INCLUDE ANY PRIZES AWARDED), HIGHER DEGREE AND FURTHER FUNDING OBTAINED AS A RESULT OF PRESENT AWARD

Publications

1. Laird A, O'Mahony FC, Nanda J, Riddick ACP, O'Donnell M, Harrison DJ, Stewart GD. Differential expression of prognostic proteomic markers in primary tumour, venous tumour thrombus and metastatic renal cell cancer tissue and correlation with patient outcome. *PLOS One*; 5 April 2013: DOI: 10.1371/journal.pone.0060483.

2. O'Mahony FC, Nanda J, Laird A, Mullen P, Caldwell H, Overton IM, Eory L, O'Donnell M, Faratian D, Powles T, Harrison DJ, Stewart GD. The use of reverse phase protein arrays (RPPA) to explore protein expression variation within individual renal cell cancers. *J Vis Exp* 2013 Jan 22; (71)); pii: 50221: DOI: 10.3791/50221.

3. Laird A, Zhong J, Ang J, Riddick ACP, Tolley DA, McNeill SA, Stewart GD. A generation of laparoscopic nephrectomy: stage specific surgical and oncological outcomes for laparoscopic nephrectomy in a single centre. *Scott Med J* 2012; 30 August; DOI: 10.1258/smj.2012.012087.

Presentations

1. Stewart GD, O'Mahony FC, Eory L, Lubbock A, Nanda J, Laird A, O'Donnell M, Riddick ACP, McNeill SA, Aitchison M, Berney D, Peters J, Rockall A, Sahdev A, Bex A, Chowdhury S, Harrison DJ, Overton I, Powles T. Renal cell cancer (RCC) heterogeneity and differential protein expression pre- and post-sunitinib therapy. 2nd Reverse Phase Protein Array World Workshop, November 2012.

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Employing a high-throughput, quantifiable proteomic approach to the development of a panel of markers predictive of outcome from sunitinib treatment in patients with metastatic renal cell cancer

2. Stewart GD, O'Mahony FC, Eory L, Lubbock A, Nanda J, Laird A, O'Donnell M, Riddick ACP, McNeill SA, Aitchison M, Berney D, Peters J, Rockall A, Sahdev A, Bex A, Chowdhury S, Harrison DJ, Overton I, Powles T. Renal cell cancer (RCC) heterogeneity and differential protein expression pre- and post-sunitinib therapy. NCRI, November 2012.

3. Laird A, O'Mahony FC, Nanda J, Riddick ACP, O'Donnell M, Meehan RR, Harrison DJ, Stewart GD. Differential protein expression in primary and metastatic renal cell cancer tissue. Cell Symposia: Hallmarks of Cancer, October 2012.

4. Laird A, Zhong J, Ang J, Riddick ACP, Tolley DA, McNeill SA, Stewart GD. A generation of laparoscopic nephrectomy: stage specific surgical and oncological outcomes for laparoscopic nephrectomy in a single centre. Scottish Urological Society 2012.

5. Stewart G, O'Mahony F, Eory L, Nanda J, Laird A, O'Donnell M, Mullen P, Riddick A, McNeill A, Aitchison M, Berney D, Peters J, Rockall A, Sahdev A, Bex A, Faratian D, Chowdhury S, Harrison D, Overton I, Powles T. Proteomic analysis of pre- and post-sunitinib treated renal cancer tissue to assess tumour heterogeneity and differential protein expression. GU ASCO, 2012.

Funding

1. MRC Scottish Clinical Pharmacology and Pathology Research Training Fellowship
June 2012; £207,097

2. Melville Trust for the Care and Cure of Cancer Research Fellowship
August 2011; £68,941

Awarded but not accepted:

1. Kidney Research UK Research Training Fellowship May 2012; £224,327

2. The Urology Foundation Research Fellowship
March 2012; £50,000

(E) ACKNOWLEDGEMENTS

I am thankful to Professor David Harrison, Mr Grant Stewart and Mr Antony Riddick for excellent supervision. I am grateful to Dr Fiach O'Mahony and Mrs Jyoti Nanda, who taught me laboratory methods and undertook much of this work with me. Dr Ian Overton, Dr Lel Eory and Mr Alex Lubbock conducted analyses of the sunitinib RPPA experimental data and survival modelling. I am also grateful to the Edinburgh Experimental Cancer Medicine Centre of Health Sciences Scotland as well as Frances Rae and Craig Marshall for assistance with construction of tissue microarrays. I am very grateful to the RCSEd Robertson Trust as well as the Melville Trust for the Care and Cure of Cancer, who provided funding for these studies.

Grant/Fellowship Reports

Role of interleukin-33 in tendon disease

Neal L Millar

Institute of Infection, Immunity and Inflammation, University of Glasgow, Glasgow, UK

ARUK Orthopaedic Clinical Research Fellowship

August 2009 to August 2011

LAY SUMMARY

Soft-tissue disorders represent the third most common orthopaedic condition in the UK, with an incidence of 18 cases per 1,000 individuals. These disorders primarily affect tendons. The most commonly affected tendons are those in the shoulder, elbow ('tennis elbow' and 'golf elbow'), knee and ankle. Key inflammatory mediators are found at significantly higher levels in and around painful tendons. However, the role of inflammation in the cascade of tendon injury is not known. Cytokines are small signalling proteins critical for mounting an immune response and play a key part in inflammatory disorders such as rheumatoid arthritis. We have demonstrated increased production of cytokines in injured tendons from patients undergoing tendon repair of the shoulder.

We found that cytokines have a significant role in tissue repair in tendon injuries. We also found that inflammation has a key role in early tendon disease in humans. Our studies have provided better understanding of the role of cytokines in tendon disease. The ultimate aim of our work is to improve/accelerate tendon healing in humans.

GRANT REPORT

IDENTIFICATION OF A SIGNIFICANT ROLE FOR INFLAMMATION IN EARLY TENDON DISEASE

To characterise the subtypes of inflammatory cells in early human tendinopathy, we explored the phenotype and quantification of inflammatory cells in samples of torn tendons and control tendons. Samples of torn supraspinatus tendons and matched intact subscapularis tendons were collected from 20 patients undergoing arthroscopic shoulder surgery. Control samples of subscapularis tendons were collected from 10 patients undergoing arthroscopic stabilisation surgery. Tendon biopsies were evaluated by immunohistochemical means by counting the number of macrophages (cluster of differentiation (CD)68 and CD206), T cells (CD3), mast cells (mast-cell tryptase) and the vascular endothelium (CD34).

Biopsies of subscapularis tendons obtained from patients with torn supraspinatus tendons exhibited significantly greater numbers of macrophages, mast cells and T cells compared with samples of torn supraspinatus tissue or control subscapularis-derived tissue ($p<0.01$). Infiltration of inflammatory cells was correlated

Role of interleukin-33 in tendon disease

inversely (r=0.5, p<0.01) with the size of tears of the rotator cuff (with larger tears correlating with a marked reduction in all cell lineages). There was a modest (but significant) correlation between the number of mast cells and CD34 expression (r=0.4, p<0.01) in matched subscapularis tendons from shoulders with supraspinatus ruptures.

We provided evidence for infiltration of inflammatory cells in early mild/moderate human tendinopathy. In particular, we demonstrated significant infiltration of mast cells and macrophages. This finding suggested a role for innate immune pathways in the events that mediate early tendinopathy.

THE ALARMIN INTERLEUKIN (IL)-33 MODULATES MATRIX CHANGES IN TENDON DISEASE

IL-33 and its receptor ST2 have become increasingly associated with musculoskeletal diseases over the past few years, and have been purported to be critical 'danger signals' in endogenous tissue. There remains a significant unmet clinical need in the understanding of tendon disorders owing largely to a lack of in-depth interrogation of the biological mechanisms underpinning the disease. However, recent studies in animals and humans have highlighted a role for inflammation.

We showed that tendons from humans and mice overexpress IL-33 if damaged, and thereby force tenocytes to undergo an early switch in collagen matrix production toward a collagen type-III phenotype via the extracellular-signal-regulated kinase/nuclear factor-kappa B signalling pathways. Moreover, administration of recombinant human IL-33 in vivo results in reduced biomechanical tendon strength at early time points. Furthermore, we highlighted a key regulatory role for the microRNA-29 family in IL-33-induced changes in the collagen matrix through direct targeting of soluble ST2. Our results, while offering new insights into the biology of IL-33/ST2 and the regulatory pathways involved in microRNA gene processing, may also assist in future strategies to treat tendon diseases.

PUBLICATIONS

1. Millar NL, Murrell GAC, McInnes IB. Alarmins in tendinopathy: unravelling new mechanisms in a common disease. *Rheumatology* 2013; 52: 769–779.

2. Millar NL, Reilly JH, Kerr SC, Campbell AL, Little KJ, Leach WJ, Rooney BP, Murrell GA, McInnes IB. Hypoxia: a critical regulator of early human tendinopathy. *Ann Rheum Dis* 2012; 71: 302–310.

3. Millar NL, Murrell GAC. Heat shock proteins in tendinopathy: novel molecular regulators. *Mediators Inflamm* 2012; 2012: 436203.

4. Zaiss MM, Kurowska-Stolarska M, Böhm C, Gary R, Scholtysek C, Stolarski B, Reilly J, Kerr S, Millar NL, Kamradt T, McInnes IB, David JP, Liew FY, Schett G. Interleukin (IL)-33 is a negative regulator of osteoclastformation and an inhibitor of bone resorption. *J Immunol* 2011 Jun 1; 186(11): 6097–6105.

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5. Hueber AJ, Alves-Filho JC, Asquith DL, Michels C, Millar NL, Reilly JH, Graham GJ, Liew FY, Miller AM, McInnes IB. IL-33 induces skin inflammation with mast cell and neutrophil activation. *Eur J Immunol* 2011 Aug; 41(8): 2229–2237.

6. Kurowska-Stolarska M, Alivernini S, Ballantine LE, Asquith DL, Millar NL, Gilchrist DS, Reilly J, Ierna M, Fraser AR, Stolarski B, McSharry C, Hueber AJ, Baxter D, Hunter J, Gay S, Liew FY, McInnes IB. MicroRNA-155 as a proinflammatory regulator in clinical and experimental arthritis. *Proc Natl Acad Sci USA* 2011 Jul 5; 108(27): 11193–11198.

7. Millar NL, Heuber AJ, Reilly JH, Xu Y, Fazzi UG, Murrell GA, McInnes IB. Inflammation is present in early human tendinopathy. *Am J Sports Med* 2010; 38: 2085–2091.

HIGHER DEGREE
Doctorate (Immunology) 2012.

ACKNOWLEDGEMENTS
I thank the Research Committee for awarding me this prestigious fellowship. It has allowed vital investigation of the inflammatory pathways in tendon disease at a world-renowned immunology laboratory. I have since taken up a clinical lecturer post in orthopaedics at the University of Glasgow.

Grant/Fellowship Reports

Role of heat-shock protein-90 in the modulation of ischemia-reperfusion injury in the kidney

Mr Stephen O'Neill

MRC Centre for Inflammation Research, Tissue Injury and Repair Group, University of Edinburgh

Maurice Wohl Research Fellowship and Small Research Support Grant

1 August 2012 to 31 May 2013

LAY SUMMARY

Kidney transplantation is the treatment of choice for patients with end-stage kidney failure. However, due to the technical process of transplantation, 10% of kidneys never work and 40% have delayed function.

Our research team seeks to identify drugs that may reduce the kidney damage caused by transplantation. We have identified a promising candidate drug that we want to study in human transplantation patients but before this can be done we need to establish exactly how the drug works.

My work began using kidney cells in the laboratory. The effect of the drug on cellular pathways causing inflammation was examined. I discovered that this drug blocked a very important pathway associated with inflammation. This was a novel and exciting finding that increased our understanding of this specific area as well as the process of inflammation in a more general way.

This work has now been extended to a mouse model of kidney injury. Using the knowledge gained from my initial experiments in cells, we have been able to identify specific inflammatory cells to investigate in mice. Confirming which inflammatory cells are affected by the drug will give a clearer indication of how to use this drug in humans, and is the focus of my ongoing research.

GRANT REPORT

(A) CLINICAL AND SCIENTIFIC SIGNIFICANCE OF ADVANCES MADE

The clinical problem

Ischemia–reperfusion injury (IRI) is an unavoidable consequence of renal transplantation. As a result of IRI, one in four kidneys obtained and transplanted from deceased donors has delayed graft function. This means that the recipient of a kidney transplant will require post-operative dialysis, which is associated with poor long-term graft function. It can also result in early graft loss, which is devastating for the patient and compounds the burden of the organ-shortage crisis. Developing a therapeutic strategy to reduce IRI (the main contributor to delayed graft function) is, therefore, highly desirable in renal transplantation, as well as being of relevance to kidneys injured by IRI in other types of surgery (e.g. cardiac, vascular).

Potential solution

Previously, our research team has shown that pre-treatment with drugs known as heat-shock protein (Hsp)90 inhibitors results in protection from renal IRI in mice. In our previous investigations, we found that an Hsp90 inhibitor called 17-Dimethylaminoethylamino-17-demethoxygeldanamycin (17-DMAG) was the most effective of these agents at reducing renal IRI. However, patients poorly tolerate older Hsp90 inhibitors such as 17-DMAG.

Role of heat-shock protein-90 in the modulation of ischemia-reperfusion injury in the kidney

This is a major problem because any agent used to reduce IRI in renal transplantation must have low toxicity. This is particularly relevant if the drug is to be given to a living donor or is to be used in a donation after cardiac death when the donor is still alive and side effects must be minimised. Therefore, one of the major challenges is to develop low-toxicity drugs that can be used safely in human renal transplantation.

Our research team, therefore, established a collaboration with Astex Pharmaceuticals to investigate an exciting new drug called AT13387. AT13387 is a novel small-molecule Hsp90 inhibitor with low toxicity in human oncology studies. Therefore, it has excellent translational potential in the context of transplantation. Currently, there is no pharmacological therapy designed to improve post-transplant function in kidneys destined for transplantation, so development of such an agent would be unique.

Why further research was needed

Although Hsp90 inhibitors had been shown to be highly effective at reducing renal IRI in mice, the mechanism of protection offered was not clear. Defining this mechanism was essential for further development of these drugs and their translation to the clinic. Moreover, if the mechanism of protection could be clarified, it was possible that newer, more highly specific agents could be designed in the future.

It was also unresolved which of the many types of inflammatory cells involved in renal IRI were targeted by Hsp90 inhibitors. This has important implications in terms of whether we should consider using these drugs in kidney-transplant donors, kidney-transplant recipients, or both. For instance, if the drug targeted a resident inflammatory cell in the kidney it would make sense to localise the protective drug to the kidney as soon as possible (i.e. by treating the donor). However, if an infiltrating inflammatory cell in the circulation was affected, it may also be highly beneficial to treat the recipient because the recipient's circulation would be carrying these cells to the kidney after the transplant. Due to the complexity of IRI, multiple cell types are involved. Therefore, we had to establish which inflammatory pathways were being influenced by these drugs to find a clue as to which cells to prioritise for further investigation.

Scientific background to the hypothesis

Hsp90 is a protein that acts as a 'chaperone' for other molecules and proteins. It has been suggested that Hsp90 might be needed to stabilise a particular enzyme complex called I kappa B kinase (IKK). Therefore, the use of drugs that inhibit Hsp90, such as 17-DMAG and AT13387, could lead to dissociation of the IKK complex. This is an important factor because IKK is required to activate a very important inflammatory mediator: nuclear factor-kappa B (NF-κB). We thought that if IKK could be disrupted then NF-κB would be repressed, which could result in a reduction in the release of pro-inflammatory cytokines and thus cellular protection in the kidney.

It has also been recognised that if cells are injured after IRI, sterile molecules called damage-associated molecular pattern molecules (DAMPS) are released. These molecules then activate an important cell surface receptor called toll-like receptor (TLR)4, which in turn can signal to

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inflammatory mediators such as NF-κB. Indeed, studies showed that TLR4 was critically involved in experimental renal IRI and contributed to IRI in human renal transplantation.

Hsp90 inhibitors have been used to treat TLR4-mediated autoimmune diseases experimentally. However, it is uncertain whether TLR4 signalling can be targeted similarly by Hsp90 inhibition in renal IRI. It is also unclear if Hsp90 inhibitors prevent TLR4-mediated injury by interrupting inflammatory signalling from TLR4 to NF-κB at the point of the IKK complex.

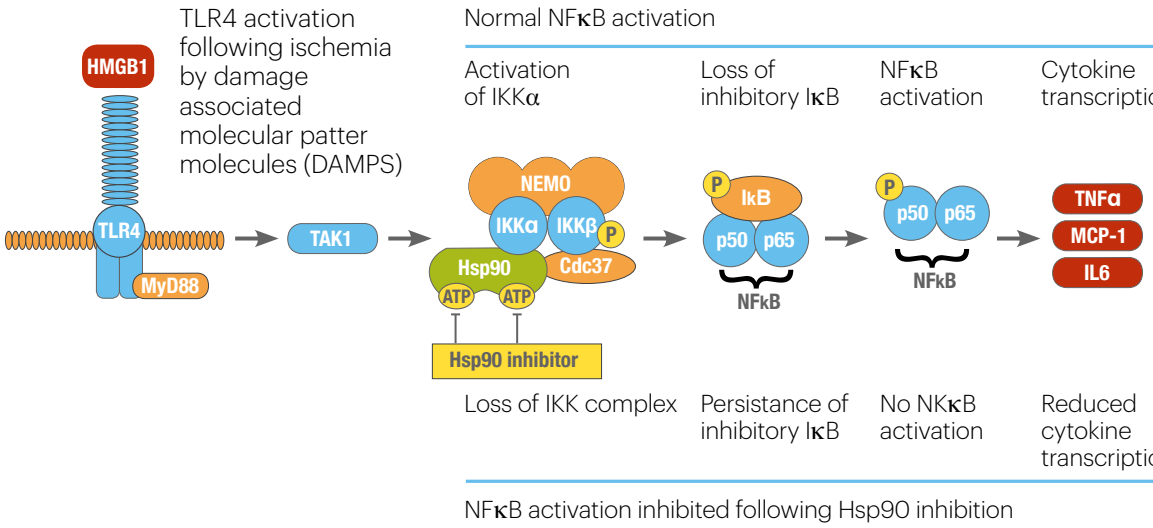
Hypothesis

My hypothesis was that Hsp90 inhibition with AT13387 would lead to a loss of the IKK complex thereby resulting in repression of TLR4 mediated NF-κB activation. This action would then lead to a reduction in the release of pro-inflammatory cytokines and, ultimately, cellular protection after oxidative stress.

Methods

Human embryonic kidney cells were stably co-transfected to express TLR4 and a secreted alkaline phosphatase (SEAP) NF-κB reporter. Cells were pre-treated with AT13387 or 17-DMAG then exposed to endotoxin-free hyaluronan (a DAMP) to stimulate sterile TLR4-specific NF-κB activation. Levels of IKKα, IKKβ and NEMO (the three main subunits of the IKK complex) were then determined by western blotting, NFκB activity by the SEAP assay, cytokine expression by array panels and cell viability after oxidative stress using a crystal violet assay.

FIGURE 1: Hypothesis of mechanism of protection offered by Hsp90 inhibition. Hsp90 inhibition may block TLR4-mediated NF-κB activation by disruption of the IKK complex.



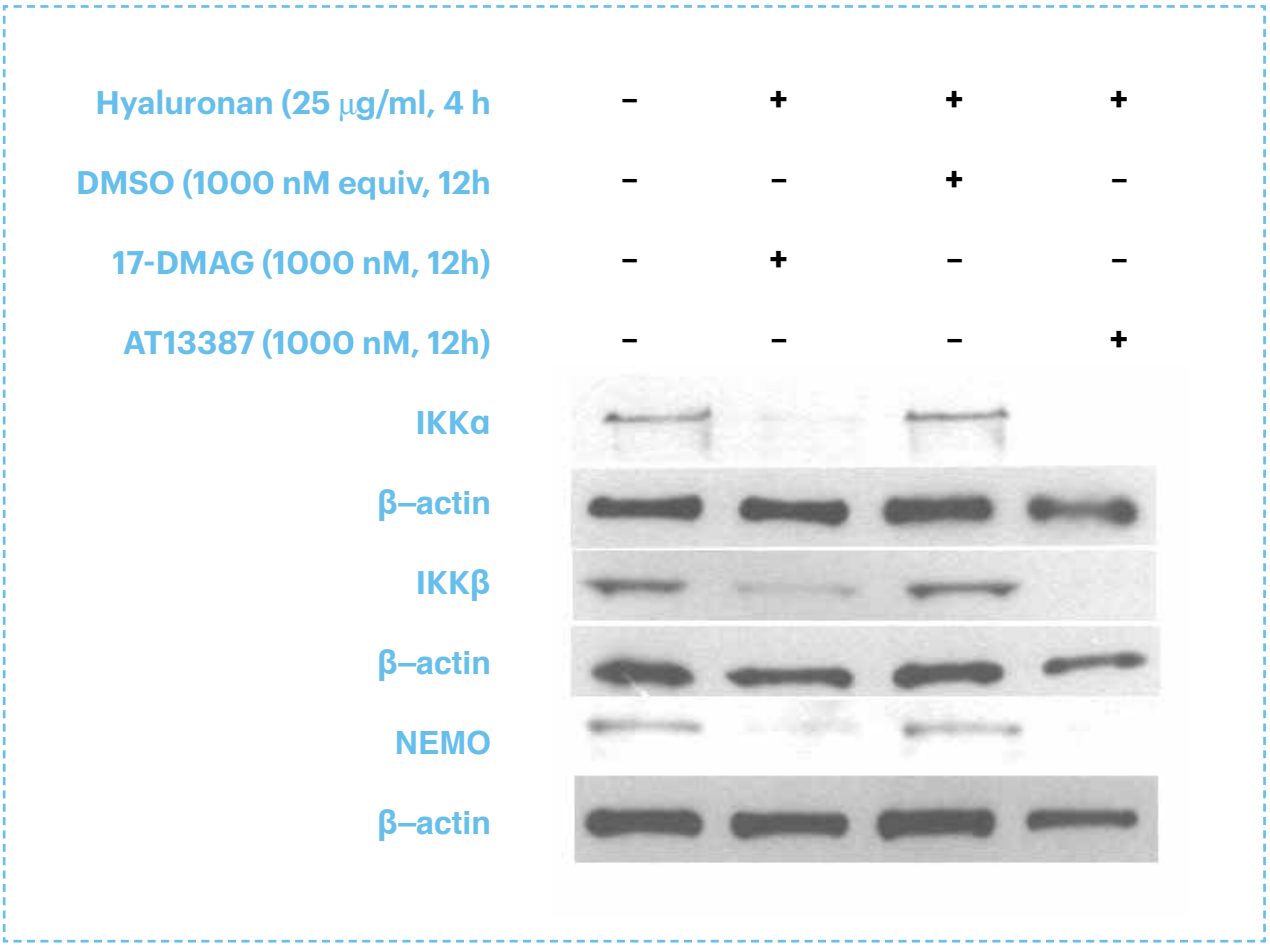
Role of heat-shock protein-90 in the modulation of ischemia-reperfusion injury in the kidney

Results

Hsp90 inhibition with AT13387 and 17-DMAG resulted in complete breakdown of IKKα, IKKβ and NEMO (figure 2).

Figure 2: IKKα, IKKβ and NEMO levels on Western blotting following AT13387 or 17-DMAG pre-treatment and hyaluronan stimulation.
This loss of the IKK complex then abolished TLR4-mediated NF-κB activation by hyaluronan (figure 3).

FIGURE 2



Role of heat-shock protein-90 in the modulation of ischemia-reperfusion injury in the kidney

Figure 3: NF-κB activity determined by SEAP assay following pre-treatment with AT13387 or 17-DMAG and hyaluronan stimulation. Results are presented from four independent experiments in a standard boxplot with individual results jittered. *p<0.05 vs. 17-DMAG, **p<0.01 vs. 17-DMAG and ***p<0.001 vs. DMSO vehicle, ANOVA.

Repression of NF-κB activation also led to a reduction in the release of pro-inflammatory cytokines and subsequently improved cell survival following oxidative stress (figure 4).

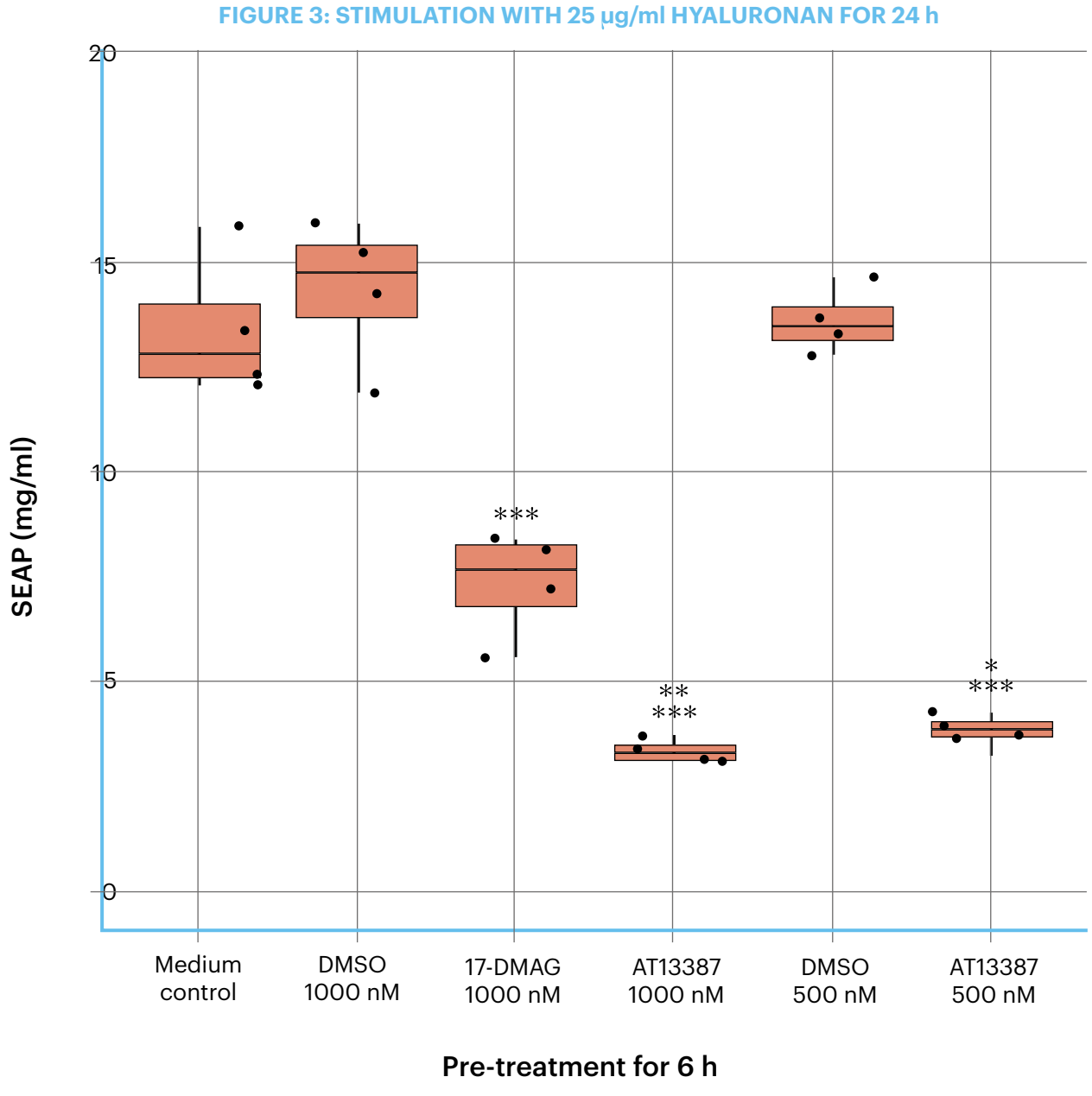


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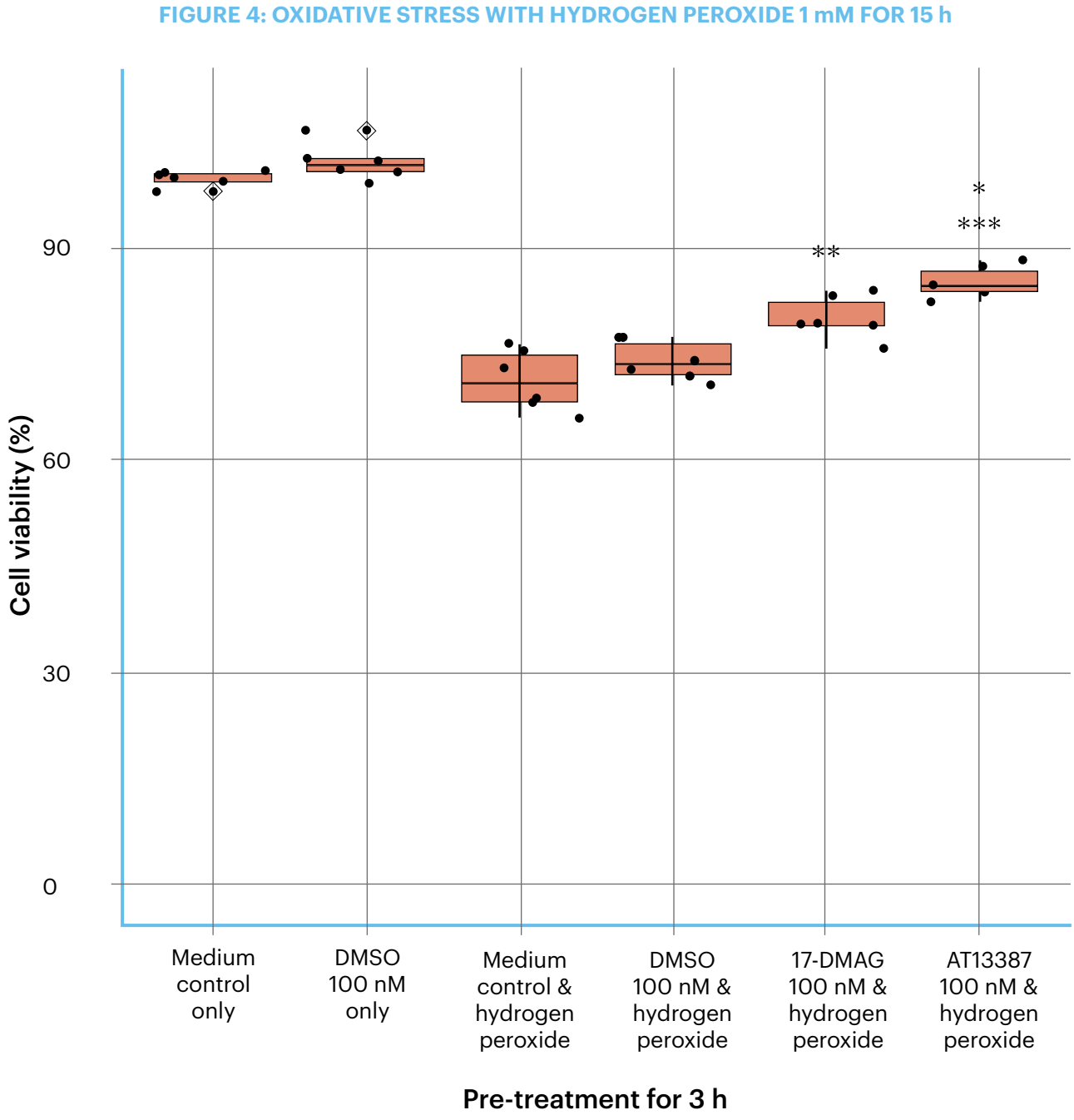
Figure 4: Crystal violet assay to determine cell viability following pre-treatment with AT13387 or 17-DMAG and oxidative stress with hydrogen peroxide. Results are presented from six independent experiments in a standard boxplot with individual results jittered. Outlier data is highlighted by a dot with a diamond in the midline. *p<0.05 vs. 17-DMAG, **p<0.01 vs. DMSO vehicle and ***p<0.001 vs. DMSO vehicle, ANOVA.



Role of heat-shock protein-90 in the modulation of ischemia-reperfusion injury in the kidney

It was then shown that breakdown of IKK by AT13387 and 17-DMAG occurred via a protein degradation process called 'autophagy'. Using an inhibitor of autophagy, called AICAR, IKK degradation by AT13387 and 17-DMAG was prevented, and partial regain of NF-κB activity observed. This "regain of function" experiment further endorsed my hypothesis.

We also found that AT13387 was more effective than 17-DMAG at reducing NF-κB activation after stimulation with hyaluronan and for improving cell survival. Although AT13387 did not appear to act through a different mechanism from 17-DMAG, it is a smaller-molecule Hsp90 inhibitor and may be more efficacious in this respect. An obvious limitation of the comparison between these two drugs is the absence of 17-DMAG *in vivo*. However, the significance of this limitation is lessened by the high toxicity and lack of translational potential of older Hsp90 inhibitors such as 17-DMAG in the context of renal transplantation.

Summary

This research has given an indication of a molecular pathway involved in the protective effect of Hsp90 inhibition in renal IRI. It has also highlighted a potential agent with potent anti-inflammatory effects that could be used for reducing renal IRI in various clinical settings. The findings are novel and add to our current understanding of the important molecular pathways involved in renal IRI and inflammatory processes in general.

Future directions

We will focus on the specific types of inflammatory cells (beginning with monocytes and macrophages) which are likely to be affected by Hsp90 inhibitors through the pathway I have identified. Identifying the role of these cell populations will give a clearer indication of whether treatment of the donor (e.g. kidney-resident macrophage cells influenced by AT13387) or recipient (e.g. circulating monocytes influenced by AT13387) is likely to be more beneficial. This is the focus of ongoing research that is supported by a Medical Research Council Clinical Research Training Fellowship. As such, my aim now that I have established how these drugs work is to ascertain how best to use agents such as AT13387 in the transplantation setting.

(B) PROBLEMS ENCOUNTERED AND STEPS TAKEN TO OVERCOME THEM

This project required me to master a range of new experimental methods: cell culture, enzyme-linked immunosorbent assay, immunohistochemistry, flow cytometry, western blotting, animal handling, microsurgery, and molecular biology. This was very challenging but, by taking the advice and availing myself of supervision from experienced researchers and technicians, I learnt these methods rapidly.

One goal of our research team is to test AT13387 in a large-animal model of transplantation. Considerable time and effort has been dedicated to securing funding for this model, and we have made it to the latter stages of three highly competitive funding processes.

Role of heat-shock protein-90 in the modulation of ischemia-reperfusion injury in the kidney

To date we have been unable to secure sufficient funding to pursue this ambition. In the near future we aim to test the drug in a murine model of transplantation instead.

However, the experience gained from these applications greatly improved my grant-writing and interview skills, which enabled me to secure further funding from a Medical Research Council Clinical Research Training Fellowship. This has allowed me to continue the mechanistic work involving monocytes/macrophages that are described in this report.

(C) COLLABORATIONS ESTABLISHED

We are working in partnership with Astex Pharmaceuticals (recently acquired by Otsuka Pharmaceutical Group) to take the novel pharmacological agent AT13387 into a human trial in renal transplantation. A material transfer agreement now exists between the University of Edinburgh and Astex Pharmaceuticals covering this work.

To take my work forward I have established collaboration with Professor Jeremy Hughes, who is now a co-supervisor for my doctorate. He is the Chair of Experimental Nephrology in Edinburgh University. His particular interest is in the role of macrophages in the injury and repair of renal tissue. He leads a dynamic research team that aims to understand more fully the diverse function of macrophages in renal disease with the goal of exploiting this for therapeutic gain. His team has led the way in the methods used to decipher the role of macrophages in renal IRI. These methods include a transgenic system that offers conditional ablation of macrophages in a mouse model of IRI, which I am now using to decipher macrophage functions in the presence of Hsp90 inhibitors. This team has also established a model of kidney transplantation in mice that AT13387 may be tested in.

(D) PUBLICATIONS AND PRESENTATIONS (INCLUDE ANY PRIZES AWARDED), HIGHER DEGREE AND FURTHER FUNDING OBTAINED AS A RESULT OF PRESENT AWARD

Publications

1. O'Neill S, Ingman TG, Wigmore SJ, Harrison EM, Bellamy CO. Differential expression of heat shock proteins in healthy and diseased human renal allografts. *Ann Transplantion* 2013; 18: 550–557.

2. O'Neill S, Hughes J. Heat-shock protein-70 and regulatory T cell-mediated protection from ischemic injury. *Kidney Int 2013; 85: 5–7.*

3. O'Neill S, Ross JA, Wigmore SJ, Harrison EM. The role of heat shock protein 90 in modulating ischemia–reperfusion injury in the kidney. *Expert Opin Inv Drug* 2012; 21: 1535–1548.

Abstracts

1. O'Neill S, Hughes J, Ross JA, Wigmore SJ, Harrison EM. Heat shock protein 90 inhibition abrogates TLR4-mediated NF-κB activity and reduces renal ischemia–reperfusion injury. *Br J Surg* 2013; 100 (S7): 2.

Role of heat-shock protein-90 in the modulation of ischemia-reperfusion injury in the kidney

Presentations

1. Edinburgh School of Surgery Prize Day 2012. Chiene Medal Session.

2. Association of Surgeons of Great Britain and Ireland (ASGBI) International Surgical Congress 2013. Moynihan Prize Session.

Prizes

1. Chiene Medal Winner 2012

Best research presentation at the Edinburgh School of Surgery Prize Day.

2. Moynihan Prize Winner 2013

Best research presentation at the ASGBI International Surgical Congress.

Higher degree

Doctorate in progress.

Further funding

1. Tenovus Scotland Small Research Grant, £9,950

2. Mason Medical Research Trust Fellowship, £50,000

3. Medical Research Council Clinical Research Training Fellowship, £13,9187

(E) ACKNOWLEDGEMENTS

• **Funding support:** RCSEd, Tenovus Scotland, Mason Medical Research Trust and Medical Research Council.

• **Technical support:** Mr Jim Black and Miss Kathryn Sangster.

Supervisory support: Mr Ewen Harrison, Professor Jim Ross, Professor Steve Wigmore and Professor Jeremy Hughes.

Travelling Grant / Fellowship Reports

Travelling Grant/Fellowship Reports

Pelvic trauma and lower-limb reconstruction/arthroplasty clinical fellowship

Andrew Douglas Carrothers
Trauma and Orthopaedics, Sunnybrook Health Sciences Centre, University of Toronto, Toronto, Canada

Cutner Travelling Fellowship in Orthopaedics

1 July 2011 to 30 June 2012

LAY SUMMARY

My overall experience in trauma and arthroplasty at the Sunnybrook Health Sciences Centre of the University of Toronto, a world-renowned academic institution, was second to none. Sunnybrook is Canada's largest level-one trauma centre and arthroplasty unit and, as such, there have been numerous clinical and academic opportunities afforded to me. I would highly commend this University of Toronto overseas clinical fellowship to any orthopaedic senior trainee as the pinnacle of his/her specialist registrar training and an excellent transition to a substantive consultant post.

My fellowship enabled wide exposure and extensive training in high-volume arthroplasty and complex polytrauma. In particular, I have extensive fellowship-level experience in the management of pelvic and acetabular fractures, peri-prosthetic fractures and lower-limb arthroplasty.

I have been involved in educational instruction throughout my professional career, from teaching to medical students to lectures at international meetings. In addition, during my fellowship year, I was an Objective Structured Clinical Examination examiner for the University of Toronto, teaching and examining orthopaedic residents at all stages of their training.

With an ongoing research portfolio encompassing basic science and clinical practice, this fellowship year I have collaborated in studies with various university departments and commercial orthopaedic companies.

GRANT REPORT

(A) CLINICAL AND SCIENTIFIC SIGNIFICANCE OF ADVANCES MADE
(A) MANAGEMENT EXPERIENCE DURING MY FELLOWSHIP

1. Leadership

(i) Sunnybrook Clinical Fellowship

Due to my Sunnybrook Fellowship I have gained unique management experience for an orthopaedic trainee:

- Micro- and macro-health economics (including the North American Major Trauma Centre Model)
- Review of tenders for clinical services
- Employment law and protocols
- Provision of clinical service
- Liaison with secondary care providers
- Planning, service procurement and audit
- Research and peer-reviewed publications
- Career planning and management
- Team-building

2. Reviewer for *J Bone Joint Surg (Br)*

- Regular reviewer for *J Bone Joint Surg (Br)* since September 2011

Pelvic trauma and lower limb reconstruction/ arthroplasty clinical fellowship

(B) TEACHING EXPERIENCE DURING MY FELLOWSHIP

1. Competency-Based Curriculum (University of Toronto)

- Mount Sinai Hospital, Toronto. October 2011 – June 2012
  Teaching and assessment of surgical residents in the Basic Surgical Skills Laboratory

2. Lecturing

- Orthopaedic Residents (including cadaveric workshops)
- Researchers and Fellows

3. Clinical teaching

- Anatomy tutorials for orthopaedic trainees
- Medical students and junior doctors (daily departmental trauma radiography/teaching; monthly audit/morbidity and mortality meetings; journal club meetings)

4. Examiner

- Surgery Clerkship and Competency-based Curriculum OSCEs, University of Toronto

(C) CLINICAL AUDIT

1. Carrothers AD, Rogers BA, Rodriguez-Elizalde S, Murnaghan JJ, Gollish J. Compliance with thromboprophylaxis using an oral factor Xa Inhibitor (rivaroxaban) after total hip and knee arthroplasty? Is oral therapy better?
- Podium presentation, Canadian Orthopaedic Association, June 2012, Ottawa.
- Holland Orthopaedic and Arthritic Centre, November 2011.

(D) ONGOING RESEARCH FROM MY FELLOWSHIP

1. Rogers BA, Carrothers AD, Stephens D, Kreder H.
Management of pelvic trauma.
CCR Submission Invitation from Professor Einhorn (Deputy Editor: Current Concepts Reviews) for *J Bone Joint Surg (Am)*.

2. Carrothers AD, Whyne C, Jenkinson RJ, Nousiainen MT, Schemitsch EH.
Do transcortical screws in a locking plate construct improve the stiffness in the fixation of Vancouver B1 periprosthetic femur fractures? A biomechanical analysis of three different plating constructs.

3. Carrothers AD, Nousiainen MT, Gollish J, Holland Orthopaedic and Arthritic Centre, Sunnybrook Health Sciences Centre.
Managing femoral sagittal plane deformity in revision total hip arthroplasty.

4. Carrothers AD, Rogers BA, Hurley RT, Jenkinson R, Stephen D, Kreder HJ.
Evaluative pilot workflow study for the use of 'gesture interface for operating room infrared imaging system' in orthopaedic fracture management.

5. Carrothers AD, Rogers BA, Hurley RT, Stephen D, Kreder HJ.
A pilot study for the implementation of intra-operative CT technology in pelvic ring instability requiring sacro-iliac screw placement.

Pelvic trauma and lower limb reconstruction/ arthroplasty clinical fellowship

6. Carrothers AD, Rogers BA, Jenkinson RJ. Patient activity levels pre and one year post total hip arthroplasty.

7. Rogers BA, Carrothers AD, Jenkinson RJ, Kreder HJ. Can the WOMAC score be used to predict patient satisfaction following hip replacement surgery? An analysis of patient-reported outcomes for joint replacement.

(E) SUBMITTED ARTICLES FOR PEER-REVIEW PUBLICATION DURING MY FELLOWSHIP

1. Carrothers AD, Rogers BA.
BMJ editorial: The implications of NCEPOD for trauma and orthopaedic surgery.
Submitted to *BMJ* May 2012.

2. Carrothers AD, Rowsell C, Jenkinson R.
Case report: Adverse reaction to metal debris in a well fixed modular metal-on-polyethylene uncemented total hip arthroplasty.
Submitted to *J Arthroplasty* May 2012.

3. Nousiainen MT, Carrothers AD.
An analysis of orthopaedic surgery fellowship training at a single academic centre: the University of Toronto experience.
Submitted to: *Can J Surg* May 2012.

4. Carrothers AD, Rodriguez-Elizalde S, Rogers BA, Razmjou H, Murnaghan D, Gollish J, Murnaghan JJ. Patient reported compliance with thrombo-prophylaxis using an oral factor Xa inhibitor (rivaroxaban®) after hospital discharge following hip and knee arthroplasty.
Submitted to *J Arthroplasty* July 2012.

All research projects, podium presentations, posters and submitted manuscripts for peer-review publication have acknowledged financial support from the RCSEd in the Cutner Travelling Fellowship.

(B) PROBLEMS ENCOUNTERED AND STEPS TAKEN TO OVERCOME THEM

As in any overseas fellowship training, it takes at least six weeks to 'find your feet'. During this time, fellows are becoming familiar with the differences in clinical practice, and fellowship supervisors are ascertaining the level of competency and training needs to be addressed. It took some time to adjust to the prescribing practices in Ontario and also to undertake all hip arthroplasties (trauma-related and primary) through the anterolateral approach and not through the posterior approach that I had been taught. I ensured that I made careful notes of drugs and the surgical steps/differences between my supervisors and other associated staff surgeons.

My fellowship was split between two locations. Cycling to and from work was pleasant during the summer months but less so during the –20°C winter and in the middle of the night while on call. However, I have returned to the UK much fitter and leaner.

(C) COLLABORATIONS ESTABLISHED

I have recently been appointed a consultant orthopaedic surgeon with a specialist interest in trauma, at Addenbrooke's Cambridge University Hospital Foundation Trust. As a research team we are already consolidating the link with Sunnybrook Health Sciences Centre for academic and

Pelvic trauma and lower limb reconstruction/ arthroplasty clinical fellowship

clinical purposes. The experience in orthopaedic polytrauma gained during my fellowship in terms of volume and complexity was second to none, as was my specialist registrar orthopaedic training in Oswestry. With the advent of Major Trauma Centres in the English and Welsh NHS there is excellent potential to collaborate in the many multinational-level randomised control trials I was involved with during my fellowship year in Toronto.

I have several ongoing research projects with the Department of Orthopaedic Surgery of the University of Toronto. I fully intend to fulfil my commitment to these projects and continue to foster links with this fine institution. One of my fellowship supervisors, Dr Markku Nousiainen, is the programme director for the University of Toronto Competency-based Curriculum for the Orthopaedic Residency Programme. It is likely that this training model will be incorporated initially in Ontario and then Canada in the foreseeable future. I will continue with our educational research in this field. I plan to continue as a reviewer for J Bone Joint Surg (Br) and wish to continue my interest in academic orthopaedics at Addenbrooke's.

(D) PUBLICATIONS AND PRESENTATIONS (INCLUDE ANY PRIZES AWARDED), HIGHER DEGREE AND FURTHER FUNDING OBTAINED AS A RESULT OF PRESENT AWARD

QUALIFICATION OBTAINED DURING MY FELLOWSHIP

- **2011–2012** Clinical Fellowship Trauma and Arthroplasty Diploma
  University of Toronto

PUBLICATIONS GARNERED DURING MY FELLOWSHIP

Peer-review publications

1. Rogers BA, Carrothers AD, Jones C. Reducing mortality for high risk surgical patients in the UK. *J Perioper Pract* 2012; 22: 167–169.

2. Rogers BA, Carrothers AD. Editorial: Using patient-reported outcome measures for assessing health-care quality. *Br J Hosp Med* (Lond) 2012 Feb; 73: 484–485.

Book chapters

1. Carrothers AD, Jenkinson RJ, Kreder H. Lower limb periprosthetic fractures: case series. Sunnybrook Health Sciences Centre, Toronto In: *AO Foundation Trauma Manual: Management of Periprosthetic Fractures*. In Press.

Published letters

1. Carrothers AD, Roach R. The pre-operative Oxford knee score alone cannot be used to rationalise timing for total knee arthroplasty or reduce the risk of patient dissatisfaction. *J Bone Joint Surg (Br),* published online, 24 February 2012. http://www.jbjs.boneandjoint.org.uk/content/93-B/12/1660/reply#jbjsbr_el_5882

2. Rogers BA, Carrothers AD. *In vitro* phenotypic modulation of chondrocytes from knees of patients with osteochondritis dissecans. *J Bone Joint Surg (Br)*, published online, 13 January 2012.

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http://www.jbjs.boneandjoint.org.uk/content/94-B/1/62/reply#jbjsbr_el_5818

Published abstracts

1. Carrothers AD, Jones P, Roach R. The Oxford knee score (OKS) is a poor predictor of outcome: An 'all-comers' 2 centre single surgeon consecutive study – is there an ideal score? Pending publication: *J Bone Joint Surg (Br)*

2. Carrothers AD, Rogers BA, Rodriguez-Elizalde S, Murnaghan JJ, Gollish J. Compliance with thromboprophylaxis using an oral factor Xa inhibitor (rivaroxaban) after total hip and knee arthroplasty? Is oral therapy better? Pending publication: *J Bone Joint Surg (Br)*

3. Carrothers AD, Gilbert RE, Jaiswal A, Richardson JB. (COA) Hip resurfacing: the prevalence of revision. Pending publication: *J Bone Joint Surg (Br)*

PODIUM PRESENTATIONS DURING MY FELLOWSHIP

International presentations

1. Carrothers AD, Rogers BA, Rodriguez-Elizalde S, Murnaghan JJ, Gollish J. Compliance with thromboprophylaxis using an oral factor Xa inhibitor (rivaroxaban) after total hip and knee arthroplasty? Is oral therapy better?
• Holland Orthopaedic & Arthritic Centre.
• Canadian Orthopaedic Association, 8–10 June 2012. Ottawa, Canada.

2. Carrothers AD, Gilbert RE, Jaiswal A, Richardson JB. Hip resurfacing: The prevalence of revision.
• Canadian Orthopaedic Association, 7–9 July 2011. St Johns, Newfoundland, Canada.

Regional presentation

1. Carrothers AD, Nousiainen MT. An analysis of orthopaedic surgery fellowship training at a single academic centre: the University of Toronto Experience.
• University of Toronto Fellowship Day, 21 June 2012. Toronto, Canada.

POSTER PRESENTATION DURING MY FELLOWSHIP

1. Carrothers AD, Rogers BA, Rodriguez-Elizalde S, Murnaghan JJ, Gollish J. Compliance with thromboprophylaxis using an oral Factor Xa inhibitor (Rivaroxaban) after total hip and knee arthroplasty? Is oral therapy better?
• 2012 World Congress on Osteoarthritis, 26–29 April 2012, Barcelona.

(E) ACKNOWLEDGEMENTS

I would like to acknowledge the RCSEd for its financial support *via* the Cutner Travelling Fellowship. I would also like to thank my fellowship supervisors at the Toronto Sunnybrook Health Sciences Centre: Dr Markku Nousiainen, Dr Richard Jenkinson and Professor Hans Kreder.

Travelling Grant/Fellowship Reports

One-year voluntary fellowship in paediatric orthopaedic surgery

James Turner
Beit Cure International Hospital, Blantyre, Malawi

Paediatric orthopaedic speciality

Cutner Travelling Fellowship

August 2012 to August 2013

LAY SUMMARY

An inspirational year was spent developing my diagnostic and surgical skills in paediatric orthopaedic surgery. I was awarded a one-year voluntary fellowship in the only hospital in Malawi that specialises in this field. Alongside outpatient and theatre sessions, I conducted regular outreach clinics in distant districts. Patients that would benefit from surgical intervention were identified and given a date to attend the Beit Cure International Hospital (BCIH), where I would undertake surgery.

The flexibility of the rota also gave me the opportunity to collect and analyse data to assess the efficacy of the Malawian Clubfoot Programme and to continue my involvement with an African multi-country trial looking at the effectiveness of the Primary Trauma Care (PTC) course.

In addition, I managed to carry out regular teaching to junior surgical colleagues at the nearby government hospital and was invited to join the college faculty on several occasions to examine undergraduate and postgraduate trainees.

My commitment to specialty and humanitarian healthcare has strengthened yet further thanks to the opportunities and positive working environment that the fellowship offered.

GRANT REPORT

(A) CLINICAL AND SCIENTIFIC SIGNIFICANCE OF ADVANCES MADE

As the international fellow at BCIH, I was given the opportunity to further my training in an internationally renowned centre of clinical excellence and research. It is the only paediatric orthopaedic institution in Malawi and, having worked with an international team, my diagnostic and surgical skills have developed exponentially and reconfirmed my desire to become a consultant paediatric orthopaedic surgeon.

In addition, I have a passion for humanitarian healthcare and, having worked in several 'third world' countries, my insight into aid to the 'developing world' has deepened no end. The hospital itself provides surgical care for children in Malawi and although the charity Cure pays in part for the work, a significant proportion of the funds that enable continued free care is generated from the private procedures we carry out on adults; hence the hospital slogan 'adults pay a fee so the kids walk for free'. It has been refreshing to be part of a healthcare institution in which all the clinical and managerial components work together to keep the patient centre-stage.

Alongside three consultant-led ward rounds, I carried out at least two outpatient clinics and spent a minimum of four sessions in theatre each

One-year voluntary fellowship in paediatric orthopaedic surgery

week. We also conducted regular outreach clinics in hospitals around Malawi. The support I received has been unsurpassed in my training to date; the consultants provide just the right amount of 'help' but give you the autonomy essential for taking that big step to consultancy. Unlike many trainee timetables in the NHS, time was allocated to research each week, and Friday afternoons were reserved for postgraduate teaching. The quality and value of these sessions (which involved trainee presentations and case-based discussions) were excellent and invaluable for FRCS revision.

B) PROBLEMS ENCOUNTERED AND STEPS TAKEN TO OVERCOME THEM

Very few problems were encountered during the year. A suboptimal aspect was the approximate time-keeping of the staff. Having worked in Africa before, this is something you have to accept despite gentle encouragement to turn up on time.

(C) COLLABORATIONS ESTABLISHED

Collaborations were established with the nearby government hospital and the College of Medicine. I was given the opportunity to instruct on courses on basic surgical skills and to lead regular tutorials with junior surgical trainees, as well as being a faculty member in several undergraduate and postgraduate college examinations.

(D) PUBLICATIONS AND PRESENTATIONS (INCLUDE ANY PRIZES AWARDED), HIGHER DEGREE AND FURTHER FUNDING OBTAINED AS A RESULT OF PRESENT AWARD

I was awarded the Diploma in Medical Care for Catastrophes in 2010. Using data that had been collected at BCIH I wrote a thesis entitled 'Impact of serial casting for clubfoot in Malawi; a conservative approach to a common deformity in sub-Saharan Africa'. It has since been published by The Royal College of Apothecaries. I have continued collecting data to substantiate evidence of the efficacy of Ponseti treatment and am analysing the data presently.

I also had the good fortune of being invited to join an Oxford University study group to look at the effectiveness of the PTC course in southern, central and eastern African countries. My involvement has taken me to Addis Ababa in Ethiopia as a representative at the annual African Surgical conference and to South Africa to finalise the data-collection forms. PTC is akin to Advanced Trauma Life Support® but aimed at resource-poor countries. A provider course is run on days one and two and, on day three, there's an Instructor course at which promising individuals identified at the beginning of the week are taught to become trainers. They then run another provider course on days four and five with the old instructors acting as mentors.

This method of conducting the PTC course ensures trainee empowerment and self-sustainability. I have directed two of the Malawian courses and plan to return when the two-year study period concludes at the end of next year. Prospective data are being collected from the nine countries and this will illustrate just how useful PTC is at improving trauma care in parts of the world where accidents and injury are endemic.

(E) ACKNOWLEDGEMENTS

- **Cutner Travelling Fellowship.**
- Mr John Cashman and the rest of the clinical and managerial staff at BCIH.

Travelling Grant/Fellowship Reports

Fellowship in trauma surgery/effect of patient fragility on outcomes after fractures of the distal radius

Samuel Molyneux

Department of Orthopaedic Trauma Surgery, Vancouver General Hospital, Vancouver, Canada

Cutner Travelling Fellowship

1 July 2012 to 31 June 2013

LAY SUMMARY

This grant helped me undertake a one-year fellowship in Vancouver, Canada as an orthopaedic trauma fellow. This is a world-renowned department and has a reputation for its excellence in training.

Vancouver General Hospital (VGH) is the tertiary referral centre for major/complex trauma for the whole of British Columbia. This is a population of more than six million – larger than the whole of Scotland. The post, therefore, provides access to a large volume of major trauma, including all aspects of upper-limb, lower-limb and pelvic surgery. In my case, it provided the perfect 'finishing school' for my chosen career in orthopaedic trauma, allowing me to build on my registrar experience and become confident in increasingly complex and difficult cases. The ethos in the department is focused on teaching, so my skills and knowledge improved dramatically. I took part in nearly 500 (often major) procedures, usually as lead surgeon. The experience gained led directly to my subsequent employment as a consultant in orthopaedic trauma surgery upon my return to the UK.

I also took part in a project looking at how patient frailty affects outcomes after distal radius (wrist) fractures. This was presented regionally and will be published internationally.

GRANT REPORT

(A) CLINICAL AND SCIENTIFIC SIGNIFICANCE OF ADVANCES MADE

This was a 'pure' trauma post and, therefore, provided a level of intensity and excellence in trauma training which is unparalleled. The skills I learnt have been directly applicable to my daily practice as an orthopaedic trauma consultant at the New Royal Infirmary of Edinburgh. Just today I carried out a new method for sacral fracture that has not been used in Edinburgh but which I learnt in Vancouver. I learnt numerous other 'tips and tricks' which I am disseminating through the department and which are helping to improve the care of patients by me and junior doctors.

I took part in a project entitled 'The effect of frailty on outcome after fracture of thedistal radius'. This is a prospective study of 250 patients aged >55 years who suffered fractures of the distal radius and who were treated at VGH. They were followed up for one year and the following were assessed: age/sex; Canadian Clinical Frailty Score; Katz Comorbidity Score and SF36 score at baseline, 12 weeks and one year; Solomon Satisfaction Score at one year; Patient-related Wrist Evaluation at one year. They also underwent

Fellowship in trauma surgery/effect of patient fragility on outcomes after fractures of the distal radius

radiography at presentation, after reduction in the emergency room, and at six weeks. I reviewed and scored the deformity on all these radiographs and this is being repeated/verified by another reviewer.

The preliminary results demonstrated that a score for patient frailty was more useful for predicting outcomes after injury than age alone, and that patient frailty has a huge impact on satisfaction, despite variations in deformity and wrist-specific functional outcome (i.e. frail patients make fewer demands on their wrists and age alone is a poor predictor of frailty). This is intuitively sensible and represents a small part of a bigger shift in thinking. Traditionally, orthopaedic research has been stratified according to age, but stratifying according to frailty would be more useful. I am setting up a study in Edinburgh based on this work in which I am attempting to predict outcomes in hip fracture based on frailty rather than age. I hope eventually to develop an improved frailty score.

(B) PROBLEMS ENCOUNTERED AND STEPS TAKEN TO OVERCOME THEM

This was a fantastic year and I would highly recommend it to anyone hoping for a career in orthopaedic trauma. The only problems were chiefly administrative – moving my entire family (wife and three children) to Canada for one year was a big upheaval. Nonetheless, I believe it was extremely beneficial for all of us. The financial burden is high, however; I have estimated that my family is ≈£65,000 worse-off for having gone abroad for one year than if we had stayed in the UK. Funding such as that provided by the Cutner Travelling Fellowship has, therefore, been vital in making this year of study possible.

(C) COLLABORATIONS ESTABLISHED

There is a long-standing collaboration between Vancouver and Edinburgh which this posting helped to solidify. Both units are seen as excellent locations for training and research and several orthopaedic trainees have travelled in each direction for their final year.

With an increase in the quality of orthopaedic research has come an increased need for international collaborative efforts in prospective orthopaedic trials. I hope to initiate such trials on a regular basis over the length of my career, and feel that the Vancouver group would be perfect to collaborate with on such work.

(D) PUBLICATIONS AND PRESENTATIONS (INCLUDE ANY PRIZES AWARDED), HIGHER DEGREE AND FURTHER FUNDING OBTAINED AS A RESULT OF PRESENT AWARD

Presentation

Molyneux SG, Lefaivre K, Guy P, Broekhuyse H, Blachut P and O'Brien PJ. Effect of fragility on outcome after distal radius fractures. Presented at the Annual Orthopaedic Research Day, University of British Columbia, March 2013.

Aiming to submit to the *J Bone Joint Surg (Am)* in the next six months.

(E) ACKNOWLEDGEMENTS

I would like to acknowledge the staff at VGH for their fantastic support during the year. In particular I wish to thank Dr Guy, Dr Lefaivre, Dr Blachut, Dr Broekhuyse and Dr O'Brien, who took such time and effort to ensure I gained the maximum possible out of the year in Vancouver.

Travelling Grant/Fellowship Reports

Fellowship in minimally invasive surgery and prevention of medical stones

Dr Keng-Siang Png
Department of Urology
Melvin and Bren Simon Cancer Center, Indiana University, IN, USA
July 2011 to June 2012

LAY SUMMARY

From July 2011 to June 2012, I was privileged to complete a one-year fellowship at the Department of Urology at the Indiana University School of Medicine under the mentorship of Professor Chandru Sundaram, Director of the Residency Program and Minimally Invasive Surgery (MIS). As the only fellow in the programme, I was involved in the full range laparoscopic and robot-assisted procedures as well as advanced endourological procedures to treat urolithiasis. At the end of one year, I had participated in more than 200 procedures and had learnt many of the finer aspects of minimally invasive urological surgery.

The second aspect of the fellowship was to train in therapy for the prevention of medical stones. Once a week, I participated in the metabolic stone-prevention clinic at the Indiana University Hospital. In addition to clinical work, I was involved in clinical research. My areas of research centred around robot-assisted partial nephrectomy and various platforms of robotic training. I was also actively involved in training urology residents in laparoscopy. I conducted bimonthly robotic training sessions for residents using the da Vinci system to undertake tasks on inanimate models. This one-year experience was extremely fulfilling and I will use my training to expand my practice at Tan Tock Seng Hospital in Singapore.

DESCRIPTION OF TRAINING

Clinical training

Being accredited by the prestigious American Endourological Society, the MIS fellowship at the Department of Urology at Indiana University met the institutional requirements of the fellowship trainee in terms of clinical volume and technology. I was mentored closely by the world-renowned laparoscopic surgeon Professor Chandru Sundaram. Besides being the MIS fellowship director, Professor Sundaram is also the university's urology residency director, so is very experienced in training young urologists. I benefited from his experience as he guided me gradually through the finer points of laparoscopy and robotic surgery. Being the only tertiary academic referral centre in Indiana, the patients referred to our unit included complex and technically challenging cases. These provided excellent training opportunities to improve my laparoscopic skills.

Another aspect of my training was the prevention and treatment of recurrent stone disease. This is a personal interest that I had

Fellowship in minimally invasive surgery and prevention of medical stones

hoped to develop. Once a week, I participated in the metabolic stone-prevention clinic at the University Hospital. This was run by Dr Sharon Moe, who is the chief of nephrology at Indiana University. Through this clinic, I learnt to manage complex cases of recurrent stone disease such as cystinuria, short-bowel disease, sarcoidosis and renal tubular acidosis. I was able to interpret Litholink® results and institute appropriate medical treatment for these patients. Upon my return to Singapore, I hope to set up a similar clinic at Tan Tock Seng Hospital to provide a multidisciplinary approach to the treatment and prevention of medical stones.

Research

There were comprehensive laparoscopic databases for prostatectomies, renal surgery and adrenalectomies. I conducted several research studies during my time at Indiana University. My areas of research centred on methods of improving robotic partial nephrectomy and validating the various platforms of robotic training. I also published several review articles in peer-reviewed journals and on medical websites. I presented my work at the World Congress of Endourology 2012 in Istanbul, Turkey, in September 2012.

I also participated in ongoing, long-term clinical trials. One of these was a randomised controlled trial studying the return of continence using a biodegradeable sling after robot-assisted laparoscopic prostatectomy. Preliminary results have been presented and patient accrual is ongoing.

Education

Being in an Accreditation Council for Graduate Medical Education-recognised residency programme, I was also closely involved in the training of urology residents at Indiana University. I functioned as a junior attending urologist and supervised residents in the operating theatre. I was also involved in the training of third- and fourth-year medical students who rotated through the programme for their month-long laparoscopy elective posting. I supervised these students in their research projects, which included database work, surgical video creation and submission for conferences and publications. I also conducted bimonthly robotic training sessions for residents. During these sessions, residents were required to undertake surgical tasks on the da Vinci system using inanimate models.

REQUIREMENTS OF TRAINEES

I had completed my medical-school training and urology residency training in Singapore. To practise in the USA as an international fellow, I

Fellowship in minimally invasive surgery and prevention of medical stones

had to obtain the United States Medical Licensing Examination as required by the Educational Commission for Foreign Medical Graduates. I also had to obtain an Indiana State medical licence from the Indiana Professional Licensing Agency. Finally, I had to undergo an interview with Professor Sundaram and required final approval from the Department of Urology of Indiana University. The credentialing process was lengthy and tedious, requiring meticulous preparation and assistance from various governing bodies in Singapore and the USA.

ADDITIONAL AWARDS AND FUNDING FOR TRAINING

Besides the John Steyn Travelling Fellowship, I was fortunate to receive additional funding for my training at Indiana University. I was awarded the Ethicon Foundation Travelling Fellowship from the Royal College of Physicians and Surgeons of Glasgow (RCPSG). This award is conferred to fellows and members of the RCPSG with good standing who are training to enhance a specialty skill that will benefit their home country.

I also received the Health Manpower Development Plan Award from the Singapore Ministry of Health in 2011. This nationwide award is given to deserving health professionals to further their training overseas in niche areas in the hope of improving the overall standard of the Singapore healthcare system.

CONCLUSION

Having returned to Tan Tock Seng Hospital in Singapore, I can now expand the repertoire of laparoscopic and robotic urological surgery at my hospital. More patients will be able to benefit from shorter hospital stays, less pain and improved outcomes of laparoscopic surgery. I will also pass on basic laparoscopic skills to our residents at Tan Tock Seng Hospital. I hope to set up a medical-stone prevention clinic in our department within the next six months for patients with recurrent stones and reduce the morbidity of stone disease. I am grateful for the support from Mr John Steyn and the RCSEd for their support in my fellowship.

Travelling Fellowship Report

Sir James Fraser Travelling Fellowship

Jimmy So
Senior Consultant Surgeon, National University Hospital
Associate Professor of Surgery, National University of Singapore, Singapore
July 2012

LAY SUMMARY

It was my privilege and honour to receive the Sir James Fraser Travelling Fellowship to visit Britain once again in July 2012. I was working as a visiting surgeon in the Royal Infirmary of Edinburgh for six months in 2004. I left with fond memories and hence it was a great occasion for me to visit Scotland again, but this time in the summer!

The first place I visited was the Oesophago-Gastric unit at the Royal Victoria Infirmary (RVH) in Newcastle, headed by Professor Michael Griffins. North-east England has a very high incidence of adenocarcinoma of the oesophagus. Under the leadership of Professor Griffins, the unit has become a world-renowned centre in surgical management of malignant and benign diseases of the oesophagus and stomach.

When I arrived I was greeted by the team and joined the ward round. The is a very high-volume surgical unit, undertaking many oesophageal and gastric resections for cancer each year. I could see the advantages of centralisation of service in this unit. The team is highly specialised and multidisciplinary. There was a wide range of procedures, ranging from minimally invasive oesophagectomy to combined organ resections. All patient data were maintained in a prospective database housed in a data centre.

I had the opportunity to visit the data centre. RVH has one of the largest databases of oesophageal cancer in the world. The database holds details of the demographic background, treatment regimens and outcomes of patients. Many interesting studies on oesophageal cancer surgery are derived from this database.

Aside from clinical work, I was inspired by Professor Griffins and his team as well as their roles in public education. The unit has established a support group for patients with oesophageal and gastric cancers. Professor Griffins has also organised a regular social campaign to promote public awareness of oesophageal cancer in the north-east of England. The campaign is impressive, including TV/radio interviews, poster displays on buses and in train stations, and even a street parade. I was amazed by the enthusiasm and the creativity of the team. My visit ended with a delicious dinner with the team surgeons. I am indebted to Professor Griffins and his team for their hospitality. It was a very fruitful and productive experience for me.

Sir James Fraser Travelling Fellowship

My next destination was the Oesophago-Gastric unit of the Royal Infirmary of Edinburgh. It was a brief visit. The unit, under the leadership of Mr Simon Paterson-Brown, has grown significantly since my last stay in 2004. It was great to visit many of my old friends and colleagues in the unit, who remain as dedicated as ever to patient care.

In Edinburgh, I had the chance to have dinner with the founder of this travelling scholarship, Mr Iain Fraser, and his wife. It was a wonderful dinner and it was my humble experience to find out about the amazing lives of his father, Sir James Fraser, and his son, Iain. I also took the opportunity to visit the beautiful Scottish highlands before heading home.

Finally, I would like to thank Ms Cathy McCartney of the College's Secretariat for her administrative support and the College for the award.

Legends

1. With Professor M Griffins at an operating theatre at the RVH
2. Visiting the Oesophago-gastric Cancer Data Centre at the RVH

WHERE ARE THE PICS FOR THIS?

Ophthalmology Grant Reports

Ophthalmology Grant Reports

Analyses of retinal digital image abnormalities seen in acute retinopathy of prematurity – is reduced oxygen therapy protective? The Benefits of Oxygen Saturation Targeting Trial II UK Retinal Image Digital Analysis (BOOST II UK RIDA) study

Professor Brian Fleck
Princess Alexandra Eye Pavilion, Edinburgh, UK
Major Grant
1 April 2011 to 31 March 2012

LAY SUMMARY

This is a three-year project. This report covers progress made during the third period of the study, from April 2011 to March 2012. The study is part of a large trial of oxygen therapy in premature babies – BOOST II UK. The aim of the trial is to determine the best amount of oxygen to use when treating these babies. One of the effects of prematurity is blindness due to retinopathy of prematurity (ROP).

Many of the study centres in the UK use specialised retinal photographs to examine babies' eyes for ROP. The Retinal Image Digital Analysis (RIDA) study has now collected all the photographs taken of babies in the BOOST II UK trial. The RIDA study comprises 145 infants, with 469 eye examinations and 2,211 retinal images. Detailed analyses are underway. Photographs will be analysed with new software that can measure retinal blood vessels automatically. This will allow us to develop software to measure the severity of ROP when babies' eyes are examined. This measure of severity will allow ophthalmologists to decide which babies need treatment for ROP, and when they need treatment, in a more reliable way than is done at present.

GRANT REPORT

(A) CLINICAL AND SCIENTIFIC SIGNIFICANCE OF ADVANCES MADE

Background

The BOOST II UK Trial is a double-blind randomised controlled trial (RCT) carried out to compare the effects of two levels of oxygen therapy on premature infants. The maximum stage of acute ROP and the need for retinal treatment are secondary outcomes during the short-term phase of the trial.

Visual disability at two years of age is a primary outcome of the trial. A significant proportion of participating ophthalmologists use digital imaging for retinal screening examinations. This is a unique feature of the trial. We have developed processes to collect these images onto a secure study server, and organise them into a format that is accessible for analyses by expert readers, and by quantification software.

Several hypotheses will be tested: infants managed with lower oxygen saturation will have more mature retinal vascularisation, and will

Analyses of retinal digital image abnormalities seen in acute retinopathy of prematurity – is reduced oxygen therapy protective? The Benefits of Oxygen Saturation Targeting Trial II UK Retinal Image Digital Analysis (BOOST II UK RIDA) study

develop less severe ROP; retinal structural and functional outcomes are influenced by ROP severity at the time of treatment, and the area of avascular retina covered by laser treatment burns; digital retinal photographs may be used for remote 'telemedicine' reading and diagnostic decisions; the severity of ROP plus disease may be quantified by analyses of computerised images, and this approach may allow more consistent decisions on diagnostic treatment to be made than at present. The collection of images has been completed, and analyses are underway.

The trial was designed to recruit 1,200 infants. An interim international meta-analysis of mortality in the two arms of the trial was done in autumn 2010. This showed a marginal (but statistically significant) reduced mortality in the higher oxygen group. The Trial Steering Committee (TSC) met and discontinued the trial on 24 December 2010. When it closed, 973 babies were enrolled in the study.

RIDA study tasks during the period covered by this report

- Catriona McIntyre-Beon has completed visits to all the study centres, and all the study images are now held on the study database.
- Mr Ken Cocker has completed the build of the server-based image database as well as all processes related to use of the database. Extensive information technology (IT) security work has been done with NHS Lothian to ensure that our processes meet the robust requirements currently in place in the NHS.
- Mr Andy King (National Perinatal Epidemiology Unit (NPEU) in Oxford) provided secure linkages between information held in the BOOST II UK trial database, and the work undertaken by Catriona McIntyre-Beon, and Mr Cocker.

(B) PROBLEMS ENCOUNTERED AND STEPS TAKEN TO OVERCOME THEM

Several IT security issues arose while using NHS Lothian's IT systems for the study. These were dealt with by Professor Fleck and Mr Cocker. This work resulted in suspension of image collection and upload in late 2011 to early 2012.

(C) COLLABORATIONS ESTABLISHED

- Close links with the NPEU in Oxford were maintained and developed further. We are planning a RCT of laser treatment versus treatment using anti-vascular endothelial growth factor for ROP.
- Closer links with the Clinical Research Imaging Centre (CRIC) in Edinburgh were developed (Dr Tom MacGillivary).
- Links with the Department of Public Health in Edinburgh (Professor Harry Campbell) were developed.
- A joint application with University College London (UCL) to the Medial Research Council for a clinical doctoral studentship has been made to undertake detailed image-analysis work in the CRIC using UCL software.

Analyses of retinal digital image abnormalities seen in acute retinopathy of prematurity – is reduced oxygen therapy protective? The Benefits of Oxygen Saturation Targeting Trial II UK Retinal Image Digital Analysis (BOOST II UK RIDA) study

(D) PUBLICATIONS AND PRESENTATIONS (INCLUDE ANY PRIZES AWARDED), HIGHER DEGREE AND FURTHER FUNDING OBTAINED AS A RESULT OF PRESENT AWARD

The BOOST TSC controls all publications from the trial. The RIDA analysis in underway but will not be published until substantive results are obtained.

Recent preliminary communications that indirectly relate to the RIDA study include:

- Stenson B, Brocklehurst P, Tarnow-Mordi W; UK BOOST II trial; Australian BOOST II trial; New Zealand BOOST II trial. Increased 36-week survival with high oxygen saturation target in extremely preterm infants. New Engl J Med 2011; 364: 1680–1682. (Professor Stenson is the Edinburgh neonatologist involved in the RIDA study).
- Fielder A for the BOOST II UK trial group. BOOST II UK Preliminary Ophthalmic Results. World Congress of ROP, Shanghai, China, October 2012 (Professor Fielder is a co-applicant on our RIDA grant).

(E) ACKNOWLEDGEMENTS

We extend our thanks to Royal Blind and the RCSEd for funding this work.

Ophthalmology Grant Reports

Preclinical testing of a new gene therapy vector for Stargardt disease

Professor Robert MacLaren
Nuffield Laboratory of Ophthalmology, University of Oxford, Oxford, UK

Major Grant

1 October 2011 to 31 September 2012

LAY SUMMARY

We are leading the only clinical trial in the world that is using an adeno-associated viral (AAV) vector to deliver a vital gene to the light-sensing photoreceptor cells in the retina. This current trial targeting the disease known as choroideraemia has been successful so far and several patients with 6/6 vision have undergone retinal gene therapy with full recovery of vision. This proves beyond any reasonable doubt that the technology may be applicable to retinal diseases before the onset of degeneration.

One of the most prevalent diseases in the UK is Stargardt disease and requires replacement of a gene known as ABCA4, which is just too large to fit into an AAV vector. The purpose of this RCSEd project was, therefore, to investigate the possibility of delivering the large ABCA4 gene in two fragments.

The project achieved its aim by the second year and the preliminary data were used in an application to the Medical Research Council (MRC) to continue this research with a £560,000

project grant. The application was successful. Hence, there has been considerable return on the initial RCSEd grant and the MRC award underlines the competitive nature of this research across all disciplines.

GRANT REPORT

(A) CLINICAL AND SCIENTIFIC SIGNIFICANCE OF ADVANCES MADE

We have split the ABCA4 coding sequence into two overlapping fragments and packaged each independently into an AAV vector. Expression of the two fragmented genes has been augmented by using a rhodopsin kinase promoter which we developed in our research team as part of this project. We also used a modified AAV capsid to increase infectivity of our vector and hence deliver more genome particles into the cell at the critical stage required for recombination into the full-length gene. We also identified ABCA4 protein in cells in tissue culture after transduction of the dual vector-packaged ABCA4. We have further characterised the ABCA4 knockout mouse in a research laboratory and a detailed manuscript has been submitted to Invest Ophthalmol Vis Sci and will be published in the near future. In that article, we submit new findings in the knockout mouse, in particular retinal flecks (which are the hallmarks of the disease in humans). We have also developed an ABCA4 assay which will be useful for detection of the protein in the retina after gene therapy.

Preclinical testing of a new gene therapy vector for Stargardt disease

(B) PROBLEMS ENCOUNTERED AND STEPS TAKEN TO OVERCOME THEM

Initially, we tried the dual-vector approach in which each transgene has only a single sequence at the inverted terminal repeat (ITR). This was the original approach described by Allocca et al. in 2008, but we found this unreliable and almost certainly it would create inconsistent transgenes because without the downstream ITR there would be no natural 'stop point' for packaging and synthesis. After considering the regulatory issues we decided to switch to the double ITR dual-vector approach and found this much more efficient because viral yields were greater.

The only negative aspect of using the dual-vector approach is that we are getting some expression of a protein from an in-frame adenine–thymine–guanine start codon in the downstream fragment. We are optimising this as part of the ongoing project by moving the sequence further from the ITR, which has intrinsic promoter activity. Otherwise this complex molecular project has progressed extremely well, as evidenced by the award of the MRC grant to take this further over the next three years.

(C) COLLABORATIONS ESTABLISHED

Professor Peter Charbel Issa is now Professor of Ophthalmology at the University of Bonn and is still collaborating on this project.

(D) PUBLICATIONS AND PRESENTATIONS (INCLUDE ANY PRIZES AWARDED), HIGHER DEGREE AND FURTHER FUNDING OBTAINED AS A RESULT OF PRESENT AWARD

Prize: ARVO CAMRAS AWARD (REM)

Publications

1. McClements ME, MacLaren RE. Gene therapy for retinal disease. *Transl Res 2013;* 161: 241–254.

2. Singh MS, Charbel Issa P, Butler R, Martin C, Lipinski DM, Sekaran S, Barnard AR, MacLaren RE. Reversal of end-stage retinal degeneration and restoration of visual function by photoreceptor transplantation. *Proc Natl Acad Sci U S A* 2013; 110: 1101–1106.

3. Lipinski DM, Thake M, MacLaren RE. Clinical applications of retinal gene therapy. *Prog Retin Eye Res* 2013; 32: 22–47.

4. Luhmann UF, Lange CA, Robbie S, Munro PM, Cowing JA, Armer H, Luong V, Carvalho LS, MacLaren RE, Fitzke FW, Bainbridge JW, Ali RR. Differential modulation of retinal degeneration by Ccl2 and Cx3cr1 chemokine signaling. *PLOS One* 2012; 7: e35551.

5. Jayaram H, Khaw PT, MacLaren RE, Limb GA. Focus on molecules: neural retina leucine zipper (NRL). *Exp Eye Res* 2012; 104: 99-100.

Preclinical testing of a new gene therapy vector for Stargardt disease

6. Lee EJ, Singh MS, Jones HE, Ahmed B, Andolina IM, Clements JT, Luong V, Munro PM, Lawton MP, Grieve KL, Aylward GW, Sillito AM, MacLaren RE. Assessment of 180 degree rotation of the choroid as a novel surgical treatment for age-related macular degeneration. *Invest Ophthalmol Vis Sci* 2012; 53: 2523–2532.

7. Charbel Issa P, Groppe M, MacLaren RE. [Gene therapy for retinal dystrophies]. *Ophthalmologe* 2012; 109: 121–128.

8. West EL, Pearson RA, Duran Y, Stipp Gonzalez A, MacLaren RE, Smith AJ, Sowden JC, Ali RR. Manipulation of the recipient retinal environment by ectopic expression of neurotrophic growth factors can improve transplanted photoreceptor integration and survival. *Cell Transplant* 2012; 21: 871–887.

9. You Q, Brown LA, McClements M, Hankins MW, MacLaren RE. Tetra-decanoylphorbol-13-acetate (TPA) significantly increases AAV2/5 transduction of human neuronal cells in vitro. *Exp Eye Res* 2012; 97: 148–53.

10. Charbel Issa P, Singh MS, Lipinski DM, Chong NV, Delori FC, Barnard AR, MacLaren RE. Optimization of *in vivo* confocal autofluorescence imaging of the ocular fundus in mice and its application to models of human retinal degeneration. *Invest Ophthalmol Vis Sci* 2012; 53: 1066–1075.

(E) ACKNOWLEDGEMENTS
I remain extremely grateful to the RCSEd, The Royal Blind, and Scottish War Blinded for this support.

Ophthalmology Grant Reports

Case–control genetic association analyses of primary rhegmatogenous retinal detachment using novel high-density exome genotyping

Aman Chandra
Moorfields Eye Hospital, London, UK

Major Grant

March 2012 to March 2013

LAY SUMMARY

Retinal detachment (RD) is an important cause of visual loss which, if left untreated, results in vision in most affected eyes deteriorating rapidly to blindness. There is evidence that RD and ocular and retinal disorders related to RD carry an important genetic component. The risk of having RD increases twofold if a sibling has the condition. Genetic variants predisposing to this common condition, however, remain unknown.

We are, therefore, investigating genetic predisposition to this condition. Following on from work funded by the RCSEd (Scottish RD Study), we have used new investigative tools to specifically define the 'coding' part of the genetic code: the exome.

This project is one of the first using this technology in any eye condition, and the very first for RD. We have completed the experimental part of the project and are now analysing the data we have obtained.

GRANT REPORT

(A) CLINICAL AND SCIENTIFIC SIGNIFICANCE OF ADVANCES MADE

We have completed genotyping on 2,000 Caucasian DNA samples collected from Scotland and London using Illumina Exome BeadChip. This was done at the Institute of Psychiatry at King's College London (KCL) in January 2013. This genotyping has been a successful, with >90% of variants on the Exome Chip called.

We have access to control data from the 1958 birth cohort (5,000 samples) and from samples genotyped as part of Generation Scotland at the University of Edinburgh (www.genetics.med.ed.ac.uk/generation-scotland/).Currently, we are undertaking quality-control analyses on these samples before full association studies.

Case–control genetic association analyses of primary rhegmatogenous retinal detachment using novel high-density exome genotyping

(B) PROBLEMS ENCOUNTERED AND STEPS TAKEN TO OVERCOME THEM

Initial difficulties revolved around where to carry out genotyping.

We investigated many research teams, both research and commercial. The KCL Institute of Psychiatry proved to be a valuable centre. They provided genotyping at a reasonable price and time. They have also allowed us access to valuable control data from the 1958 birth cohort.

Analysing rare variants in such association studies is challenging, as is assessing for population stratification. We are, therefore, very fortunate to have access to the Generation Scotland data, which were analysed using the same chip. The patients in this study are from the same geographic background as our samples.

(C) COLLABORATIONS ESTABLISHED

As a direct result of this grant, collaborations have been established to collect samples toward replication of findings from this study. These collaborations are with the following units:

- Guy's and St Thomas' Hospital, London (KCL), UK
- Department of Ophthalmology, Addenbrooke's Hospital, Cambridge, UK
- Calgary Retina Consultants, Calgary, Alberta, Canada
- Royal Victorian Eye and Ear Hospital, Melbourne, Australia

(D) PUBLICATIONS AND PRESENTATIONS (INCLUDE ANY PRIZES AWARDED), HIGHER DEGREE AND FURTHER FUNDING OBTAINED AS A RESULT OF PRESENT AWARD

Data from this study are being incorporated into a doctoral thesis (Aman Chandra) with publications to follow.

There has been acknowledgement of this grant in the following publications:

- Chandra A, Banerjee PJ, Charteris DG. Grading in ectopia lentis (GEL): a novel classification system. *Br J Ophthalmol* 2013; 97: 942–943.
- Kirin M, Chandra A, Charteris DG, Hayward C, Campbell S, Celap I, Bencic G, Vatavuk Z, Kirac I, Richards AJ, Tenesa A, Snead MP, Fleck BW, Singh J, Harsum S, Maclaren RE, den Hollander AI, Dunlop MG, Hoyng CB, Wright AF, Campbell H, Vitart V, Mitry D. Genome-wide association study identifies genetic risk underlying primary rhegmatogenous retinal detachment. *Hum Mol Genet* 2013; 22: 3174–3185.

(E) ACKNOWLEDGEMENTS

- RCSEd
- Royal Blind

Ophthalmology Grant Reports

Role of TGFβ and its receptors on the proliferation and differentiation of human Müller stem cells obtained from retinectomy specimens

David G Charteris
Research and Development, Vitreo-retinal Surgery Unit, Moorfields Eye Hospital, London, UK

Small Research Grant

April 2012 to April 2013

LAY SUMMARY

The light-sensitive layer lining the back of the eye (retina) contains cells (Müller stem cells (MSCs) or Müller glia) which, in zebrafish, can regenerate into new functioning nerve cells to replace damaged ones. MSCs are present in humans but, rather than regenerating into new functioning cells, they produce scar tissue in the diseased retina. Evidence suggests that a molecule, transforming growth factor (TGF)β may have a role in inhibiting the ability of human MSCs to regenerate into new cells.

We studied 16 specimens from 16 patients undergoing surgery to reattach their retinas (retinectomy specimens). We attempted to isolate MSCs from these samples and have tried to grow these cells in culture with and without TGFβ. Initial experiments demonstrated promise, showing cells with the distinctive appearance and shape of MSCs growing well in culture. However, so far, we have been unable to immortalise these cells and retain a population of 'true' stem cells.

GRANT REPORT

(A) CLINICAL AND SCIENTIFIC SIGNIFICANCE OF ADVANCES MADE

Clinical advances

We have perfected the method of retrieval of retinectomy samples by aspirating directly from the back of the vitrectomy cutter. This has allowed three main advantages:

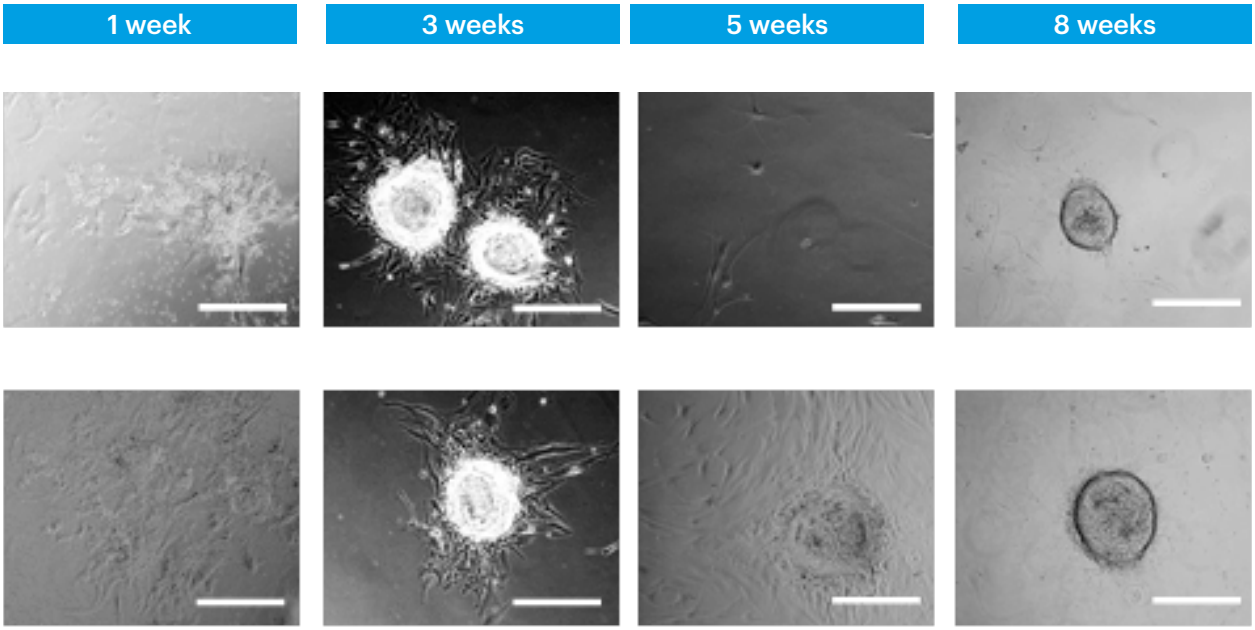
1. Insignificant interruption of the routine surgical procedure.
2. Ability to retrieve retinectomy samples with high initial cell counts.
3. Superior mechanical 'digestion' of retinal tissue, thereby reducing the need for repeated enzymatic digestion.

Scientific advances

Ten retinectomy specimens from nine patients were studied initially without TGFβ inhibition, and a further six specimens were treated with TGFβ inhibitory factors. After one week of culture, cells with the expected morphology of human

Role of TGFβ and its receptors on the proliferation and differentiation of human Müller stem cells obtained from retinectomy specimens

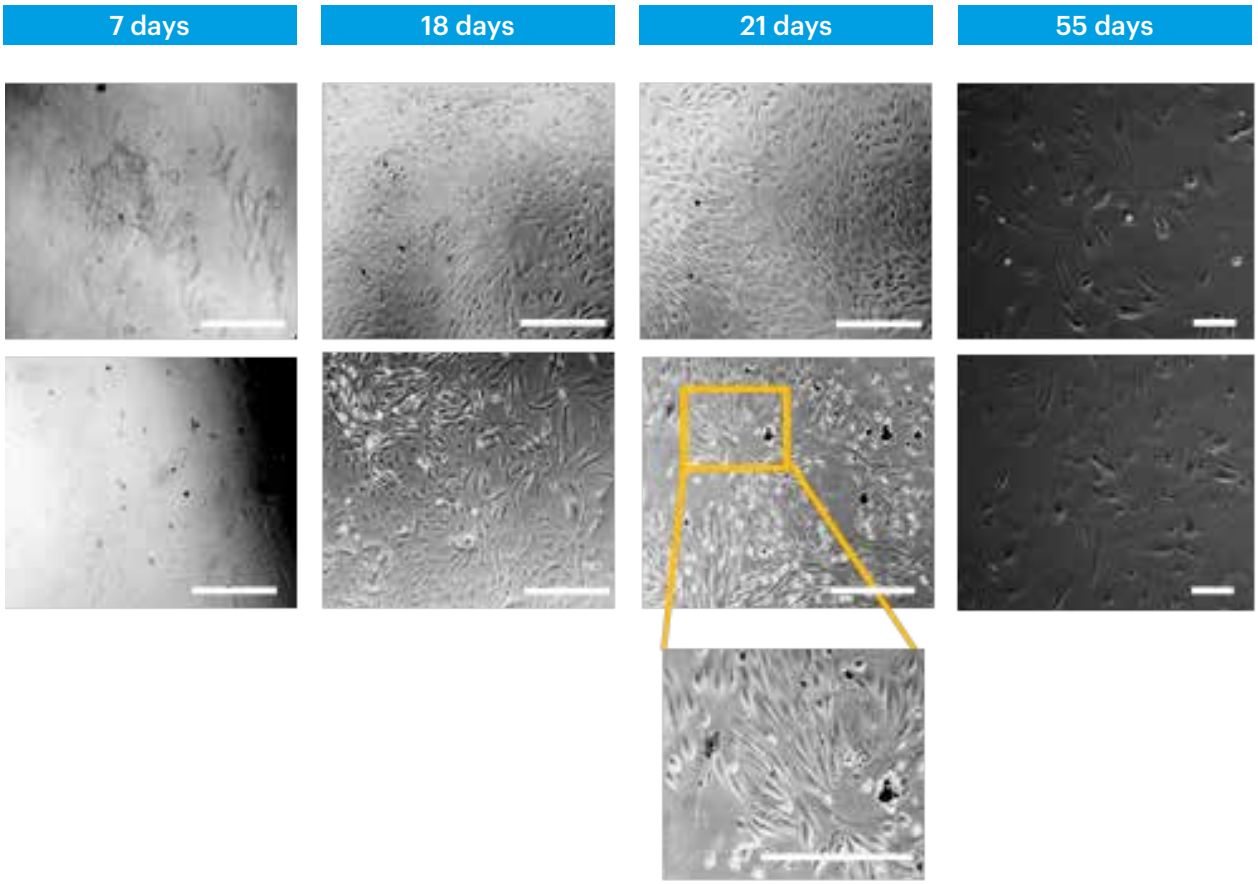
FIGURE 1



MSCs were observed in all processed samples, with higher yields achieved in samples derived from the cutter. They appeared bright when viewed under a contrast microscope and showed characteristic glial morphology. For the first ten samples, cells appeared to proliferate and form colonies by week-1. Most, however, survived up to eight weeks and appeared to form neurospheres (Figure 1). Attempts to release cells from these spheres and encourage proliferation were unsuccessful.

Role of TGFβ and its receptors on the proliferation and differentiation of human Müller stem cells obtained from retinectomy specimens

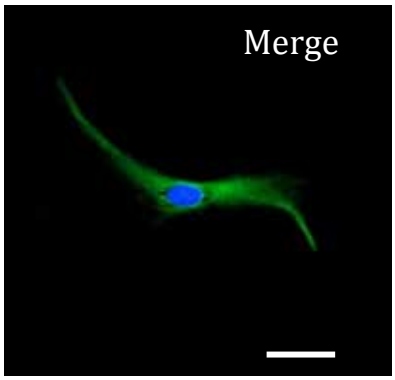
FIGURE 2



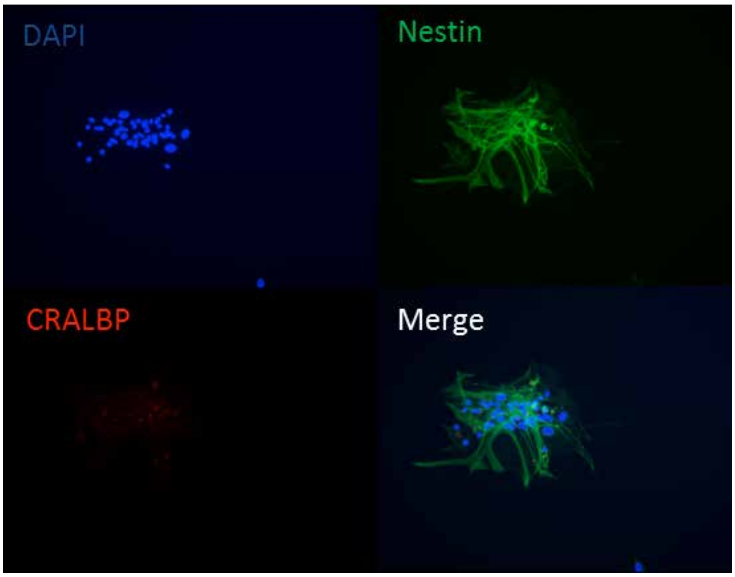
However, two cutter-derived samples appeared to proliferate and survived up to four passages and expansions into larger plates (Figure 2). Unfortunately, immortality was not achieved because eventually all cells adopted the morphology of terminally differentiated mature Müller glia with a characteristic flattened, enlarged and elongated cell morphology by day-55.

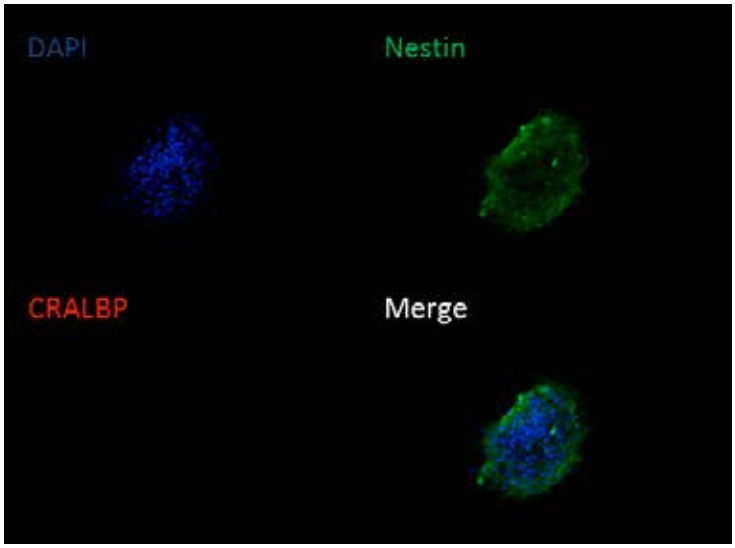
Role of TGFβ and its receptors on the proliferation and differentiation of human Müller stem cells obtained from retinectomy specimens

◁ **FIGURE 3**



Cells harvested by forceps and cutter retrieval methods from one adult donor were stained for the neuroprogenitor marker nestin and the specific marker of Müller glial cells cellular retinaldehyde-binding protein (CRALBP). Cells were removed from culture at one, four and seven days after primary plating. Figure 3 shows a cell staining positive for nestin on day-1, a colony of cells at day-4 staining positive for nestin and CRALBP, and cells forming a neurosphere at day-7.





▷

Role of TGFβ and its receptors on the proliferation and differentiation of human Müller stem cells obtained from retinectomy specimens

(B) PROBLEMS ENCOUNTERED AND STEPS TAKEN TO OVERCOME THEM

Using established protocols, it was possible to isolate cells with the appropriate morphology of human MSCs which survived up to ten weeks in culture. However, only two of the 16 specimens processed proliferated to reach confluence and were expanded into larger tissue-culture plates. Successful culture of human MSCs from surgical specimens have reported initial cell counts of <106, yielding an efficiency of 40%. This value increases to 77% with higher cell counts (2×106). Samples retrieved in this study were small fragmented pieces of tissue, so achieving such high cell numbers was difficult. This may explain the lack of success in this study so far, and is consistent with the observed higher cell yield with cutter-derived samples when compared with those retrieved with forceps.

When specimens were cultured with the addition of TGFβ inhibitory factors, there was no effect on increasing cell yield, with an apparent overall reduction in proliferation seen. No samples proliferated to confluence when treated with TGFβ inhibitory factors. Reports in rodents describe a cytostatic effect of mature retinal neurons attributed to the effect of TGFβ signalling. Inhibition through a combination of the type-II receptor of TGFβ (TGFβRII) and a fragment crystallisable (Fc) protein and a pan-TGFβ blocking antibody has been shown to enhance the ability of Müller glia to re-enter the cell cycle in response to epidermal growth factor (EGF). The lack of effect observed in this study may also be explained by the low initial cell counts at the time of plate seeding. Additionally, the cocktail of inhibitory factors used included a small-molecule inhibitor of TGFβRI called SB-431542. In effect, with the combined inhibition of TGFβRI and TGFβRII–Fc, the entire TGF pathway may have been inhibited, thereby resulting in 'excessive inhibition' of proliferation per se, with a lack of selectivity. Therefore, selective inhibition of TGFRs may yield different effects on cell proliferation and it would be interesting to examine this hypothesis.

All retinectomy samples were retrieved from eyes with proliferative vitreoretinopathy (PVR). Activation of glial cells and reactive gliosis is a key feature in PVR, confirmed by the marked staining for glial fibrillary acidic protein (GFAP) noted in the specimens in this study. It is likely that a large proportion of the resident population of glial cells had already become activated and terminally differentiated towards reactive gliosis. Hence, it is logical to assume that the population of cells with stem-cell abilities in this tissue may have already been depleted. Nevertheless, the positive staining for neuroprogenitor markers in retrieved retinectomy specimens and in cells early in culture suggests that the population of Müller glia with stem-cell characteristics are not completely absent in this dystrophic tissue.

Two samples initially showed signs of proliferation in which cell-growth arrest at ≈7–8

Role of TGFβ and its receptors on the proliferation and differentiation of human Müller stem cells obtained from retinectomy specimens

weeks was observed. Hence, future work may involve the study of local factors that inhibit proliferation at this stage. Sequential western blot analyses of cells in culture, or proteomics of the culture media at specific time points, may help to identify the changes in protein production that occur through an initial proliferative period followed by a rapid cessation and terminal differentiation.

Ideally, peripheral biopsies from human retinas would be large enough to obtain sufficient primary cell counts and 'healthy' enough to harbour a significant population of Müller glia with stem-cell characteristics. In practice, this is difficult to achieve becausee patients undergoing vitreoretinal surgery have, by definition, vitreoretinal disease. Higher yields may be obtained from anterior retinectomies from large giant retinal tears of recent onset, thereby achieving adequate initial cell counts from tissue in which reactive gliosis is less marked.

(C) COLLABORATIONS ESTABLISHED

Professors Steve Fisher and Geoff Lewis,
Neuroscience Research Institute,
University of California, Santa Barbara, CA, USA

(D) PUBLICATIONS AND PRESENTATIONS (INCLUDE ANY PRIZES AWARDED), HIGHER DEGREE AND FURTHER FUNDING OBTAINED AS A RESULT OF PRESENT AWARD

The funded project was included in a successful MPhil-to-doctorate upgrade for co-investigator Philip Banerjee and further work will constitute a substantial proportion of the final doctoral thesis.

(E) ACKNOWLEDGEMENTS

I gratefully acknowledge funding from RCSEd and Royal Blind.

Ophthalmology Grant Reports

Optimisation of ABCA4 gene expression as a treatment for Stargardt disease

Professor Robert MacLaren
Nuffield Laboratory of Ophthalmology, University of Oxford, Oxford, UK

Major grant

1 October 2012 to 31 September 2013

LAY SUMMARY

The project represented the second year of a study designed to develop gene therapy for Stargardt disease. Stargardt disease is the most common inherited retinal degeneration and presents in childhood (also known as 'childhood macular dystrophy') and is caused by a deficiency of a gene known as ABCA4. This gene is quite large and slightly outside the maximum size possible for delivery with the adeno-associated viral (AAV) vector commonly used in retinal gene therapy. The purpose of the RCSEd project was, therefore, to investigate the possibility of delivering the large ABCA4 gene in two fragments.

The project achieved its aim by the second year and the preliminary data were used in a successful application to the Medical Research Council (MRC) to continue this research with award of a £560,000 Developmental Pathway Funding Scheme. The first milestone of that MRC grant has been met and there is now a clear path towards gaining regulatory approval. The achievement of MRC follow-on funding highlights the clinical application of the award as well as the high quality of the science presented because these MRC awards are highly competitive.

GRANT REPORT

(A) CLINICAL AND SCIENTIFIC SIGNIFICANCE OF ADVANCES MADE

ABCA4 has now been split successfully and recombined in vivo and in vitro after transduction of dual AAV vectors, each containing an overlapping fragment. The ABCA4 protein has been expressed in vivo in the ABCA4 knockout mouse and in human embryonic kidney (HEK) cells. The correct protein size has been confirmed by western blotting. The phenotype of the ABCA4 knockout mouse has been characterised in detail.

(B) PROBLEMS ENCOUNTERED AND STEPS TAKEN TO OVERCOME THEM

Some truncated ABCA4 protein was expressed from the downstream vector. We, therefore, met with the Medicines and Healthcare Products Regulatory Agency in London in October 2013 to obtain some informal advice about whether or not this would be acceptable in a clinical trial. We were informed that we should undertake some toxicity studies on the retina of mice but that, in general, there would be no concerns as long as there were no detrimental effects from the protein.

Optimisation of ABCA4 gene expression as a treatment for Stargardt disease

(C) COLLABORATIONS ESTABLISHED

Professor Peter Charbel Issa is now Professor of Ophthalmology at the University of Bonn and is still collaborating on this project. His doctoral thesis, which includes much of this work, was submitted in October 2013.

(D) PUBLICATIONS AND PRESENTATIONS (INCLUDE ANY PRIZES AWARDED), HIGHER DEGREE AND FURTHER FUNDING OBTAINED AS A RESULT OF PRESENT AWARD

1. Lipinski DM, Barnard AR, Charbel Issa P, Singh MS, De Silva SR, Trabalza A, Eleftheriadou I, Ellison SM, Mazarakis N, MacLaren RE. VSV-G and VEEV-G pseudotyped lentivirus vectors differentially transduce corneal endothelium, trabecular meshwork and human photoreceptors. *Hum Gene Ther* 2014; 25: 50–62.

2. Charbel Issa P, Barnard AR, Singh MS, Carter E, Jiang Z, Radu RA, Schraermeyer U, MacLaren RE. Fundus autofluorescence in the ABCA4-/- mouse model of Stargardt disease – correlation with accumulation of A2E, retinal function and histology. *Invest Ophthalmol Vis Sci* 2013; 54: 5602–5612.

3. Tolmachova T, Tolmachov OE, Barnard AR, de Silva SR, Lipinski DM, Walker NJ, Maclaren RE, Seabra MC. Functional expression of Rab escort protein 1 following AAV2-mediated gene delivery in the retina of choroideremia mice and human cells *ex vivo*. *J Mol Med (Berl)* 2013 Jul; 91(7): 825–837.

4. Charbel Issa P, De Silva SR, Lipinski DM, Singh MS, Mouravlev A, You Q, Barnard AR, Hankins MW, During MJ, Maclaren RE. Assessment of tropism and effectiveness of new primate-derived hybrid recombinant AAV serotypes in the mouse and primate retina. *PLoS One* 2013; 8: e60361.

5. Charbel Issa P, Groppe M, MacLaren RE. [Gene therapy for retinal dystrophies]. *Ophthalmologe* 2012; 109: 121–128.

6. You Q, Brown LA, McClements M, Hankins MW, MacLaren RE. Tetra-decanoylphorbol-13-acetate (TPA) significantly increases AAV2/5 transduction of human neuronal cells *in vitro*. *Exp Eye Res* 2012; 97: 148–153.

7. Charbel Issa P, Singh MS, Lipinski DM, Chong NV, Delori FC, Barnard AR, MacLaren RE. Optimization of *in vivo* confocal autofluorescence imaging of the ocular fundus in mice and its application to models of human retinal degeneration. *Invest Ophthalmol Vis Sci* 2012; 53: 1066–1075.

(E) ACKNOWLEDGEMENTS

I gratefully acknowledge funding from the RCSEd and Royal Blind.

King James IV Professorship Lectures

King James IV Oration 2013

King James IV Professorship Lectures

Single-event multilevel surgery in spastic diplegia: evidence and outcomes

Professor H Kerr Graham

13 September 2012

British Orthopaedic Association Annual Congress

Manchester, 11–14 September 2012

INTRODUCTION/SUMMARY

Single-event multilevel surgery (SEMLS) is a complex surgical/rehabilitation intervention to improve gait and functioning in ambulant children with cerebral palsy, and it has been practised for more than 20 years. In a systematic review, we found that the evidence for SEMLS was of poor quality because of the lack of controlled trials, limited choice of outcome measures and short-term follow-up1. In an effort to address deficiencies in the evidence base, we conducted and reported the first randomised controlled trial (RCT) of SEMLS, followed by a prospective cohort study of the five-year outcomes of the patients enrolled in the RCT2,3. More recently, we have reported the outcomes of SEMLS from a large, retrospective study using outcome measures utilised in the RCT4. A comparison of the results from the RCT and a retrospective cohort study carries important lessons for the design of future clinical trials.

What is SEMLS?

There are many competing terms for the complex surgical interventions used in children with spastic diplegia, to correct deformity, improve gait and functioning. After the seminal work from pioneers such as Mercer Rang and James Gage, the concept of sagittal plane balance (SPB) became central to the management of gait in children with cerebral palsy, dictating that surgical interventions should not be at a single level but at multiple anatomic levels (hip, knee, ankle, foot). With this philosophy and approach, the problems of single-level surgery (e.g. precipitating crouch gait) might be avoided and improved postoperative SPB achieved. In some discussions, the *rationale* for SEMLS is often quoted as reducing the number of hospitalisations and periods of rehabilitation[1]. This is of course true, but the primary biomechanical reason is establishment of SPB. The benefits of reduced hospitalisation and reduced healthcare costs are secondary to this biomechanical principle.

The descriptive terms for surgery at multiple levels that have been used in the literature include 'multilevel surgery', 'multiple lower extremity procedures' and SEMLS[1]. We introduced and prefer the term SEMLS given that it emphasises the concept of undertaking all the procedures during one surgical intervention and that these interventions will be at multiple anatomical levels. For practical purposes, we defined SEMLS as surgery on at least two anatomical levels, on both lower limbs, although the surgical indication did not need to be symmetric. This was an attempt to

Single-event multilevel surgery in spastic diplegia: evidence and outcomes

define a minimum amount of surgery for SEMLS and to allow collation of data from the more extensive surgical programmes for analytical and comparison purposes. This definition is, of course, quite arbitrary but has been found to be useful in subsequent clinical trials and systematic reviews[1,2].

What are the technical aspects of multilevel surgery?

In children with spastic cerebral palsy, the growth of muscle tendon units lags behind the growth of the corresponding long bones. Over time, complex musculoskeletal disease develops, including contractures of the two joint muscles, torsional deformities in long bones and instability of joints (including the hip joint and the mid-foot). This has been described as 'progressive musculoskeletal disease'. It is this musculoskeletal disease that is amenable to correction by orthopaedic surgery in the context of SEMLS. Clearly it would be preferable to correct the abnormality in the motor cortex. Although many centres around the world are exploring trials of stem cells, no effective reparative biological therapies have been reported for the brain lesion in cerebral palsy. SEMLS, therefore, refers to the correction of fixed musculoskeletal deformities (including muscle recessions, tendon lengthening, tendon transfers, rotational osteotomies) and stabilisation of unstable joints (including the hip and the mid-foot).

The number of procedures can be 4–24. Our long-term average number of procedures in a SEMLS surgery is eight (SD 4). We have developed a strong commitment to 'dose-based surgery' in which we not only identify the deformity requiring correction from a biomechanical assessment (including three-dimensional gait analyses) but also seek to arrive at the appropriate 'surgical dose'. For example, more than a dozen procedures have been described for the correction of equinus deformity, and each of these equates to a different surgical dose. New methods and new implants are improving the reliability and functional outcomes of individual surgical procedures that comprise SEMLS.

How can we measure the outcome of SEMLS?

The measurement of gait and function in ambulant children with cerebral palsy is challenging. The most useful approach is based on a full biomechanical assessment combined with functional scales and measures of health-related quality of life (HR-QoL)[1,2]. In our routine surgical work this involves three-dimensional gait analyses, a standardised physical examination by an experienced physiotherapist, and physiological testing such as measurement of the Physiological Cost Index or the energy expenditure of walking. Classification scales and functional scales include the Gross Motor Function Classification System (GMFCS), the Functional Mobility Scale and the Functional Assessment Questionnaire. HR-QoL scales include the Child Health Questionnaire (Australian version) and the Paediatric Orthopaedic Data Collection Instrument (PODCI)[2,3].

Single-event multilevel surgery in spastic diplegia: evidence and outcomes

What is the evidence for SEMLS?
Given our ambition to improve the evidence base for SEMLS, we embarked on a systematic review of published evidence1. This study found that the design and reporting of SEMLS studies were improving with the development of multidisciplinary teamwork and frameworks such as the World Health Organization's International Classification of Function (WHO-ICF). However, the evidence base was limited by a lack of RCTs, long-term follow-up, and standardisation of outcome measures. The need for a summary statistic of gait was emphasised.

What is the Melbourne evidence for SEMLS?
In an effort to address some of these deficiencies in the literature, the team at the Hugh Williamson Gait Laboratory, Royal Children's Hospital Melbourne, embarked on the world's first RCT of SEMLS for children with spastic diplegia. Despite application to national and international funding bodies, and excellent external reviews, we were unable to secure external grant funding for a large multicentre RCT, which had been our original goal. However, based on prior sample size calculations from observed change in cohort studies, we consider that a single-centre pilot RCT might still be a significant help in establishing the evidence for SEMLS and in providing a framework to guide large, multicentre RCTs in the future. In this study, 19 children (12 boys and seven girls, mean age, nine years and eight months) with spastic diplegia (all GMFCS II or III) were randomised to SEMLS or a control group. The control group underwent a programme of progressive resistance strength training and the surgical group proceeded to SEMLS, with a raft of outcome measures obtained at baseline one, two and five years postoperatively. In keeping with the suggestions from our systematic review, we included a summary statistic of gait, the Gait Profile Score (GPS), as well as a published index, the Gillette Gait Index (GGI). Secondary outcome measures were the Gross Motor Function Measure (GMFM-66), the Functional Mobility Scale, time spent in the upright position (Uptimer) and a HR-QoL questionnaire (Child Health Questionnaire)[2,3].

In this group of children, 85 surgical procedures were undertaken for a mean of eight procedures per child (SD 4). The surgical group had a 34% improvement in the GPS and a 57% improvement in the GGI at 12-month follow-up. The control group had a small, non-significant deterioration in both indices. The between-group differences for the change in the GPS (–5.5; 95% confidence interval, –7.6 to –3.4) and the GGI (–218; –299 to –136) were highly significant. Although no changes were found in secondary outcome measures, a small (but clinically and statistically increase) in GMFM-66 of 4.9% was recorded two years after surgery. This study provides level-1 evidence that SEMLS results in clinically and statistically significant improvements in gait function with smaller improvements in

Single-event multilevel surgery in spastic diplegia: evidence and outcomes

gross motor function and HR-QoL. During the design and conduct of this trial, we recognised the impossibility of retaining children with spastic diplegia in a RCT for >12 months on ethical and practical grounds. This was based on the finding that the control group deteriorated within the 12-month time frame. We decided to address this problem in two ways.

After conclusion of the RCT, the children in both groups (control and surgical) proceeded to annual review in the Gait Laboratory and long-term follow-up with a further report of outcomes five years after commencement of the trial3. This study was published in 2011 and the findings were very encouraging. The measured improvements in gait function and gross motor function persisted at five-year follow-up with a measured improvement in the GPS of 5.29°, which is more than three-times the Minimal Clinically Important Difference. Gross motor function had shown a slight deterioration but was still clinically and statistically better than baseline with a mean improvement of 3.3%[3].

The final piece of evidence from our department was a retrospective analysis of 121 children with spastic diplegia who had SEMLS between 1995 and 2008. We were interested in two main issues: identification of factors predictive of good outcomes and assessment of outcomes in a large group of children in non-trial conditions. The GPS was again used as the primary outcome measure and the mean improvement was 4.3°, which compared very favourably with 4.6° in the RCT at one year and 5.3° at five years[2–4]. The strongest predictor of outcome was the baseline or pre-operative GPS. This finding is important for understanding the benefits and limitations of SEMLS. It has relevance to pre-operative planning, counselling of parents and the design of clinical trials.

SEMLS: the future?

There is a need for large, multicentre trials of SEMLS with a balanced raft of outcome measures addressing all domains of the WHO-ICF. However, the mean improvement in GPS was similar in the retrospective study and in the RCT[2,3,4]. This finding begs the question as to whether RCTs are in fact necessary in determining the outcome of SEMLS. The GPS, based as it is on accurate and reliable instrumented gait analyses, is essentially immune to bias. It may be sufficiently sensitive and objective to obviate the need for RCTs. Prospective trials with clearly identified entry criteria, data collection with appropriate outcome measures at specified intervals and longer-term follow-up may be a superior methodology for SEMLS than a time-limited RCT.

References

1. McGinley J, Dobson F, Ganeshalingam R, Shore BJ, Rutz E, Graham HK. Single-event multilevel surgery for children with cerebral palsy: a systematic review. *Dev Med Child Neurol* 2012; 54: 117–128

Single-event multilevel surgery in spastic diplegia: evidence and outcomes

2. Thomason P, Baker R, Dodd K, Taylor N, Selber P, Wolfe R, Graham HK. Single-event multilevel surgery in children with spastic diplegia: A pilot randomized controlled trial. *J Bone Joint Surg* 2011; 93-A: 451–460.

3. Thomason P, Selber P, Graham HK. Single-event multilevel surgery in children with bilateral spastic cerebral palsy: A 5 year prospective cohort study. *Gait Posture* 2013; 37: 23–28.

4. Rutz E, Donath S, Tirosh O, Graham HK, Baker R. Explaining the variability improvements in gait quality as a result of single-event multilevel surgery. *Gait Posture* 2013; 38: 455–460.

King James IV Professorship Lectures

Studies on cellular and molecular control of impaired wound healing: the development of novel nanomedicine approaches to 'old wounds'

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Wound healing represents a complex series of inter-related (but overlapping) processes[1]. The work of our research team in the last 20 years has been to: understand the cellular and molecular control of these events; identify the mechanisms of impaired dermal healing; develop new diagnostic tests and therapies for patients with non-healing, chronic skin wounds (which affect >3% of the UK population aged >65 years).

Initially, studies within our research team were used to define and characterise the role of fibroblast phenotypes in mediating the preferential healing of oral wounds. We showed that oral fibroblasts could preferentially migrate into experimental wounds and reorganise extracellular matrices compared with their dermal counterparts[2,3]. We demonstrated that this was in part due to the increased production of hepatocyte growth factor and activation of matrix metalloproteinase-2 by oral fibroblasts[4,5], and showed the role of the homeobox gene PRX-2 in controlling these events[6]. In chronic non-healing skin wounds we showed a distinct (but contrasting) metalloproteinase phenotype of fibroblasts from the wound bed[7,8]. To characterise the differences between oral and dermal fibroblasts, we established a longitudinal patient-matched study of oral and dermal fibroblasts; demonstrating the importance of 'molecular ageing' in these processes and how fibroblasts from the oral mucosa have an extended in vitro lifespan suggestive of progenitor cell populations[9,10], which represent an alternative source for the generation of progenitor cell populations from the oral mucosa[11].

In dermal wound healing, we showed how alteration in fibroblast phenotypes could influence many of the disease-associated changes[12] and, using expression profiling methods, we went on to demonstrate the 'genetic signature' of fibroblasts from the chronic wound bed and a potential role of chronic inflammation in driving fibroblast senescence and telomeric erosion[13]. Fibroblast differentiation within healing wounds *in vivo* is characterised by acquisition of the myofibroblast phenotype, and we showed how the oral cells resisted the 'ageing' and transforming growth factor (TGF)-β-induced myofibroblast transformation that this was associated with altered hyaluronan metabolism[14–17].

We knew something of the altered extracellular matrix (ECM) remodelling in cells from chronic non-healing wounds, but we sought to determine the role of wound bacteria in influencing these processes[18,19]. We undertook the first prospective detailed characterisation of the microflora of chronic wounds[20–25] and went on to show how bacteria colonising these wounds are distinct from the same bacteria that colonise patient-matched uninjured skin (in expression of virulence factors and immunostimulatory ability[26]),with healing being associated with local decreases

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in toll-like receptor (TLR)-2- and TLR-4-inducing responses[27]. Relatively little was known of the microflora of chronic wounds, so over-prescription of antibiotics was common[28,29]. In an attempt to avoid this scenario, we developed novel diagnostic tests to deliver immediate information on the bacteria within wounds[30–33].

Even with this information, the bacteria in chronic wounds are present in a biofilm and are an ideal environment for the exchange of genetic material and acquisition of antimicrobial resistance[34]. Part of this resistance is mediated by the biofilm itself (a dense mixture of bacteria and extracellular polymeric substance (EPS)), which modifies cellular behaviour and responses to treatment[35]. Developing three-dimensional models of these interactions *in vitro* enabled us to study these processes and test potential therapies, including those we developed later in the research team[36–38].

At the start of our research, it was widely perceived that application of external modifiers of biological responses (e.g. growth factors) perceived to be lacking in the wounds would revolutionise the management of scars or chronic wounds. History proved this to be a hugely expensive failure, which reflects the 'hostile' nature of the wound environment, with proteases and free-oxygen radicals[39]. In an attempt to overcome this problem, we worked to develop a new generation of growth-factor conjugates to avoid degradation of modifiers of biological responses. These are growth factors chemically conjugated to a biodegradable polymer. We developed the world's first polymer therapeutics for wound healing[39] and showed the ability of these epidermal growth factor (EGF)–dextrin conjugates to modify wound-healing responses *in vitro* and *in vivo*[40–42]. This technology is moving forward in our research team to support stem-cell applications in nerve repair[43].

We have employed polymer therapeutics to address the problems of bacterial biofilms and antimicrobial resistance. In the treatment of multidrug resistant (MDR) infections we have invented novel polymers. We have used our knowledge of polymer therapeutics to develop the first of a new generation of novel 'nano-antibiotics'. We applied the principles we used with EGF in wound healing to 'coat' and covalently link the antibiotic colistin (which is highly effective in treating MDR infections but whose use is severely limited due to its toxicity) with dextrin to create a novel antibiotic[44]. In this work we demonstrated how conjugation reduces toxicity so that these nano-antibiotics can revolutionise the way we treat infections.

In our most significant research translation in novel polymer therapies, working with Algipharma AS, we invented novel biopolymers based on modification of the naturally occurring biopolymer alginate. Alteration of the normal repeating G-M composition enables it to interact with the EPS in bacterial biofilms. We have shown how it potentiates the action of antibiotics against MDR bacteria such as *Acinetobacter* species by up to 500-fold[45]. These compounds have now completed phase-1 studies[46] and phase-2a studies in the UK and EU.

The potential of OligoG to treat MDR infections in injured military personnel has led to a joint award from the US Department of Defence. This award, together with an EU Eurostars award, has allowed us to move toward clinical utilisation to the treatment of injured military servicemen and young adults with cystic fibrosis.

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References

1. Thomas DW et al. *J Oral Maxillofac Surg* 1995; 53: 442–448.
2. Stephens P et al. *J Dent Res* 1996; 75: 1358–1364.
3. Stephens P et al. *J Perio* 1997; 82: 163–169.
4. Stephens P et al. *Wound Rep Regen* 2001; 9: 35–44.
5. Stephens P et al. *Br J Dermatol* 2001; 144: 229–237.
6. White P et al. *J Invest Dermatol* 2003; 120: 135–144.
7. Cook H et al. *J Invest Dermatol* 2000; 115: 225–233.
8. Wall S et al. *J Invest Dermatol* 2002; 119: 91–98.
9. Enoch S et al. *J Dent Res* 2009; 88: 916–921.
10. Enoch S et al. *J Dent Res* 2010; 89: 1407–1413.
11. Davies LC et al. *Stem Cells Dev* 2010; 19: 819–830.
12. Stephens P et al. *Exp Cell Res* 2003; 148: 22–35.
13. Wall R et al. *J Invest Dermatol* 2008; 128: 2526–2540.
14. Meran S et al. *J Biol Chem* 2007; 282: 25687–25697.
15. Simpson RL et al. *Am J Pathol* 2007; 175, 5: 1915–1928
16. Meran S et al. *J Biol Chem* 2008; 283: 6530–6534.
17. Simpson RM et al. *Am J Pathol* 176, 3: 1215–1228.
18. Wall IB et al. *Wound Rep and Regen* 2002; 10: 346–353.
19. Stephens P et al. *Brit J Dermatol* 2003; 148: 456–466.
20. Davies C et al. *Wound Rep and Regen* 2001; 9: 332–341.
21. Hill KE et al. *J Med Micro* 2002; 51: 1–9.
22. Hill KE et al. *J Med Micro* 2003; 52: 365–369.
23. Davies CE et al. *J Clin Microbiol* 2004; 42: 3549–3557.
24. Andersen A et al. *J Wound Care* 2007; 16: 171–174.
25. Davies CE et al. *Wound Rep Regen* 2007; 15: 17–22.
26. Emanuel CE et al. *J Clin Micro* (submitted).
27. Pukstad BS et al. *J Dermatol Sci* 2010; 59: 115–122.
28. Howell-Jones RS et al. *Wound Rep Regen* 2006; 14: 387–393.
29. Howell-Jones RS et al. *J Antimicrob Chemother* 2005; 55: 143–149.
30. Moseley R et al. *Br J Dermatol* 2004; 150: 401–413.
31. Moseley R et al. *Wound Rep Regen* 2004; 12: 419–429.
32. Wildeboor D et al. *Eur J Clin Micro Infect Dis* 2012; 10: 1553–1556.
33. Wagstaffe S et al. *Anal Chem* (in press).
34. Percival S et al. *Wound Rep Regen* 2012; 19: 1–9.
35. Hill K et al. *J Antimicrob Chemother* 2010; 65: 1195–1206.
36. Malic S et al. *Oral Micobiol Immun* 2007; 22: 188–194.
37. Malic S et al. *Microbiology* 2009; 155: 2603–2611.
38. Malic S et al. *J Wound Care* 2011; 12: 569–574.
39. Hardwicke J et al. *J Controlled Release* 2008; 130: 275–283.
40. Hardwicke J et al. *Mol Pharmaceutics* 2010; 7: 699–707.
41. Hardwicke J. *Br J Ophthalmol* 2010; 94: 1566–1570.
42. Hardwicke J et al. *J Controlled Release* 2011; 152: 411–417.
43. Ferguson EL et al. *Int J Pharmaceutics* 2010; 402: 95–102.
44. Thomas D, Walsh T, Ferguson E. Therapeutic Conjugates. GB 1010500.5. UK Patent (2010).
45. Onsoyen E, Myrvold R, Thomas DW, Walsh T. (GB 0909556.3 GB 0909529.0 PCT/GB2010/001098 PCT/GB2010/001096 PCT/GB2010/001097)
46. Clinicaltrials.gov identifier: NCT00970346.

King James IV Oration 2013

Oral *Candida* in health and disease

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INTRODUCTION

Candida species are opportunistic fungal pathogens residing principally in the human gastrointestinal tract (including the oral cavity), causing disease when host defences are impaired[1,2]. These diseases range from superficial infections of the oral and vaginal mucosae, to life-threatening systemic infections that can spread via the blood stream to organs throughout the body. The mechanisms that entail transformation of the commensal *Candida* into a disease causing parasitic existence are incompletely understood.

As about one-half of the human population harbour *Candida* species[3,4], mainly *Candida albicans*. Demystification of the pathogenesis of mucosal and systemic candidiasis (synonym: candidosis) would be of value in the prevention and management of these relatively common ailments, especially in compromised population groups, such as those infected with the human immunodeficiency virus (HIV).

Here I summarise our findings on the myriad pathogenic mechanisms of an enemy that 'sleeps with us' ordinarily, yet strikes with a vengeance given an opportunity. A sketch of the key areas of research is listed below:

I. Clinical epidemiology of *Candida* in healthy and diseased population groups around the world and, for the first time, in most Asian cohorts.

2. Emerging *Candida* species, such as *C. krusei, C. glabrata, C. parapsilosis* and *C. tropicalis* as well as characterisation of their pathogenic attributes in the context of mucosal colonisation.

3. Basic pathogenic mechanisms of *Candida* species appertaining to colonisation, including: adhesion mechanisms and biofilm formation; hydrophobicity; production of phospholipase and proteinase; formation of germ tubes.

4. Non-specific immune factors in the host, such as the salivary constituents lactoferrin and lysozyme as well as the epithelial–fungal interface in the pathogenesis of candidiasis.

5. Clinicopathological features of oral candidiasis, including classification and characterisation of variants, such as chronic atrophic candidiasis, chronic hyperplastic candidiasis, angular cheilitis and HIV-associated oral candidiasis.

6. Role of antifungal agents and biocides in oral candidiasis.

I. Clinical epidemiology of oral *Candida* and *enterobacteriaceae*

Data from various groups (including those with diabetes mellitus (DM), those on cytotoxic therapy, hospitalised patients, comatose patients, and stroke sufferers), from disparate

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geographic regions (including Cambodia, China, Thailand, Tanzania, Tibet, UK) suggest that previous information on the prevalence of oral candidal carriage of 40–60% derived essentially from Western cohorts cannot be extrapolated to Asia[4]. Our findings indicate that the prevalence is relatively low in healthy Asians (14–40%). Also, for the first time, we defined the prevalence of oral carriage of *enterobacteriaceae* in many of the foregoing cohorts. *In vitro* studies on the interactions between *Candida* and *enterobacteriaceae* have revealed the: (i) enigmatic, oral co-existence between *Candida* and *enterobacteriaceae*; (ii) lethal growth-suppressant effect of the latter on the former; (iii) necessity to categorise *enterobacteriaceae* as resident (rather than transient) oral commensals, particularly in ethnic Chinese populations[5].

2. Emerging *Candida* species

Comprehensive characterisation of various phenotypic, genotypic and proteomic attributes of emerging pathogens – *C. krusei, C. glabrata, C. parapsilosis* and *C. tropicalis* from a multitude of isolates from Asia and Europe have uncovered the adenine auxotrophic heterozygosity and the genomic heterogeneity in *C. krusei*[6–9]. Pioneering investigations in animals confirmed the increased expression of *C. krusei* virulence in compromised states but not in healthy states and, in other studies, its relatively high susceptibility to human lactoferrin and most intriguingly its high oral prevalence in leprosy patients in Thailand. These have reconfirmed the necessity for *C. krusei* to be reassigned into another genus[6].

Examination of isolates of *C. parapsilosis* and *C. glabrata* from diverse origins using novel molecular ultrastructural and proteomic technology revealed a remarkable degree of genetic diversity, including polymorphism of chromosome length and kinetics of biofilm growth. Interestingly, the superficial isolates compared with the systemic isolates of *C. parapsilosis*, demonstrated markedly enhanced adhesion to host surfaces and increased cell-surface hydrophobicity, implying evolutionary trends related to eco-diversity in *Candida* species[7].

3. Pathogenic mechanisms of *Candida* species

Candidal adhesion to host surfaces and subsequent development into biofilms is an essential prerequisite for infection. Pioneering methods that we developed in the early 1980s to explore candidal adhesion to epithelial and denture acrylic surfaces and subsequent biofilm development have demystified the complexity of this phenomenon[10–12] and, in particular, characterised the:

- enhanced candidal adherence and biofilm development on oral surfaces due to dietary carbohydrates and the clinical implication of such a diet on candidiasis[11];
- conflicting role of whole saliva and serum and sub-therapeutic concentrations of antifungals and biocides on modulating candidal adhesion, and biofilm formation[13];
- drug resistance of candidal biofilms likely to be due to its inherent antioxidative capacity[14];
- role of indigenous bacteria, particularly coliforms in inhibiting and fostering yeast adhesion and biofilm formation[15]; and
- suppression of expression of human beta-defensin on epithelia due to hyphal invasion16[16].

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4. Non-specific immune factors in hosts

The interactions of non-specific oral immune factors and *Candida* are poorly understood. *In vitro* exposure of pathogenic *Candida* species to salivary proteins, lysozyme and lactoferrin reflected their natural hierarchy of oral prevalence because *C. albicans* and *C. glabrata* were the most resistant to these proteins[17]. Their susceptibility decreased further if they were pre-cultured in sucrose. In contrast, pre-exposure to low concentrations of antifungal agents resulted in increased sensitivity to lactoferrin. Although salivary lysozyme and lactoferrin are likely to act synergistically *in vivo*, this was not the case *in vitro*. Additionally, non-specific defences in murine bronchial fluid were candidacidal, but mainly for non-*albicans* species[18]. Taken together, these results have elucidated (to some degree) the role of salivary antifungal defences and probable reasons for the predominance of *C. albicans* in the oro-pharynx.

5. Clinicopathological features of oral candidiasis

With the HIV epidemic, novel variants of oral candidiases were described, throwing into disarray the conventional classification of disease. Hence, a new improved classification, which has now received worldwide acceptance, was proposed in which the disease entities are categorised principally as 'primary' and 'secondary' oral candidiasis, with further new subdivisions thereafter[19].

Management of the clinical variants of oral candidiasis poses new challenges. Epidemiological surveys among the young and elderly in differing geographic locations indicated a relatively highly prevalent, yet poorly managed, disease. Angular cheilitis is an often-ignored, peri-oral variant of candidosis. Pioneering work in Asians confirmed its dual, candidal-staphylococcal aetiology and ultrastructure[20].

Next, we described the microbe–host interactions in two compromised groups: those with DM and those infected with the HIV. Exploration of the salivary (blood group) secretor status and candidal infection in DM cases revealed a higher prevalence of oral carriage of *Candida* in secretors.

An extensive enquiry into the oral manifestations of Asian and African cohorts infected with the HIV resulted in a new database highlighting the features of salivary defences and secondary oral diseases that may differ from Western populations[21,22]. Other novel findings relevant to HIV disease include: (i) identification of global sub-types of oral *C. albicans*[23]; (ii) characterisation of *Candida*-associated palatal papillary hyperplasia[24] as well as the ultrastructure of pseudomembranous candidiasis; (iii) enhanced avidity of *Candida* to the buccal cells of HIV carriers[25].

6. Role of antifungal agents and biocides

Antimycotic therapy in oral candidiasis was evaluated in another series of studies with emphasis on the post-antifungal effect and its impact upon candidal physiology. Finally, novel modes of antifungal delivery and therapeutic applications were examined together with management of oral candidiasis in compromised patients[26].

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Conclusions

The findings detailed above have redefined the clinical epidemiology and complex behavioural patterns of *Candida* species within the oral habitat of humans, and how it interacts with the host. These and related data have led to a deeper understanding of the prevention and management of mucosal candidal infections in general and oral candidiasis in particular.

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References

1. Samaranayake LP, MacFarlane TW (Eds). *Oral Candidosis*. Wright-Butterworth, London, UK, 1990.
2. Samaranayake YH, Samaranayake LP. Experimental oral candidiasis in animal models. *Clin Microbi Rev* 2001; 14: 398–429.
3. Samaranayake LP. *Essential Microbiology for Dentistry*, 4th Ed. Churchill Livingstone, Edinburgh, 2012.
4. Samaranayake LP. Commensal oral *Candida* in Asian cohorts. *Int J Oral Sci* 2009; 1: 2–5.
5. Sedgley C, Samaranayake LP. Oropharyngeal prevalence of *enterobacteriaceae* in humans: a review. *J Oral Med Oral Path* 1994; 23: 104–113.
6. Samaranayake YH, Samaranayake LP. *Candida krusei*: biology, epidemiology, pathogenicity and clinical manifestations of an emerging pathogen. *J Med Microbiol* 1994; 41: 295–310.
7. Luo G, Samaranayake LP. *Candida glabrata*, an emerging fungal pathogen, exhibits superior relative cell surface hydrophobicity and adhesion to denture acrylic surfaces compared with *Candida albicans*. *APMIS* 2002; 110: 601–610.
8. Dassanayake RS, Samaranayake LP. Characterization of the genetic diversity in superficial and sytemic human isolates of *Candida parapsilosis* by randomly amplified polymorphic DNA. *APMIS* 2000; 108: 153–160.
9. Samaranayake YH, Wu PC, Samaranayake LP, Ho PL. The relative pathogenicity of *Candida krusei* and *Candida albicans* in the rat oral mucosa. *J Med Microbiol* 1998; 47: 1047–1057.
10. Samaranayake LP, MacFarlane TW. An *in vitro* study of the adherence of *Candida albicans* to acrylic surfaces. *Arch Oral Biol* 1980; 25: 603–610.
11. Samaranayake LP, MacFarlane TW. The effect of dietary carbohydrates on the *in vitro* adhesion of *Candida albicans* to human epithelial cells. *J Med Microbiol* 1982; 15: 511–517.
12. Nair R, Samaranayake LP. The effect of oral commensal bacteria on candidal adhesion to human buccal epithelial cells. *J Med Microbiol* 1996; 179–185.
13. Ellepola ANB, Samaranayake LP. The effect of limited exposure to antimycotics on the relative cell surface hydrophobicity and the adhesion of oral *Candida albicans* to buccal epithelial cells. *Arch Oral Biol* 1998; 43: 879–887.

Oral *Candida* in health and disease

14. Seneviratne CJ, Wang Y, Jin L, Abiko Y, Samaranayake LP. Proteomics of drug resistance in *Candida glabrata* biofilms. *Proteomics* 2010; 10: 1444–1454.

15. Bandara HM, Yau JY, Watt RM, Jin LJ, Samaranayake LP. *Pseudomonas aeruginosa* inhibits *in-vitro Candida* biofilm development. *BMC Microbiol* 2010; 10: 125.

16. Lu Q, Jayatilake JAMS, Samaranayake LP, Jin L. Hyphal invasion of *Candida albicans* inhibits the expression of human β-defensins in experimental oral candidiasis. *J Invest Dermatol* 2006; 126: 2049–2056.

17. Anil S, Samaranayake LP. Impact of lysozyme and lactoferrin on oral *Candida* isolates exposed to polyene antimycotics and fluconazole. *Oral Dis* 2002; 8: 199–206.

18. Samaranayake LP, Tobgi RS, MacFarlane TW. Anti-*Candida* activity of murine broncho-alveolar lavage fluid. *J Med Microbiol* 1994; 40: 350–357.

19. Samaranayake LP, Yaacob H. Classification of oral candidosis. In: Samaranayake LP and MacFarlane TW (eds) *Oral Candidosis*. Wright Butterworth, London, 1990, 124–132.

20. Warnakulasuriya KAAS, Samaranayake LP, Peiris JSM. Angular cheilitis in a group of Sri Lankan adults: a clinical and microbiologic study. *J Oral Pathol Med* 1991; 20: 172–175.

21. Samaranayake LP, Scully C. Oral candidosis in HIV infection. (Leader) Lancet 1989; ii: 1491–1492.

22. Samaranayake LP. Oral mycoses in human immunodeficiency virus infection: a review. *Oral Surg Oral Med Oral Pathol* 1992; 73: 171–180.

23. Tsang PCS, Samaranayake LP et al. Biotypes of oral *Candida albicans* isolates from diverse geographic locations. *J Oral Pathol Med* 1995; 24: 32–36.

24. Reichart P, Schmidt-Westhausen A, Samaranayake LP, Philipsen HP. *Candida*-associated palatal papillary hyperplasia in human immunodeficiency virus infection. *J Oral Pathol Med* 1994; 23: 403–405.

25. Tsang PCS, Samaranayake LP. Factors affecting the adherence of *Candida albicans* to human buccal epithelial cells in human immunodeficiency virus infection. *Br J Dermatol* 1999; 141: 1–8.

26. Ellepola ANB, Samaranayake LP. Oral candidal infections and antimycotics. *Crit Rev Oral Biol Med* 2000; 11: 172–198.