THE ROYAL COLLEGE OF SURGEONS OF EDINBURGH

Research Report 2014–2016

FROM HERE, HEALTH

Contents

Foreword from the President

Over the past decade, The Royal College of Surgeons of Edinburgh has dedicated millions of pounds towards surgical research. This has become an increasingly significant resource to students and researchers as medical research budgets come under ever-increasing pressure. Each year, the College invites applications for Research Fellowships, grants and bursaries. These range in scope and scale, from three-year full-time research posts jointly supported by the College and the Medical Research Council (MRC) to eight-week vacation 'research tasters' for medical students.

Michael Lavelle-Jones, President, RCSEd

Most awards continue to be made to practising surgeons, but almost all the reports illustrate the inter-disciplinary nature of surgical research. Invariably, our award-holders express their gratitude to the scientists with whom they have collaborated on their projects. However, it is clear that the surgeon brings an invaluable clinical perspective to the team and that this is recognised by their collaborators. Whether they return to full-time surgical practice or follow a specifically academic path (some have gone on to careers as 'clinician scientists'), almost without exception award-holders have gained enormously from the experience of being involved directly in research. The College is rightly proud of the contribution it has been able to make (and is still making) to the advance of clinical practice through research.

None of this would be possible without the generosity of our supporters. Trusts, foundations and individuals have played important parts in allowing surgeons to innovate and explore possibilities that ultimately allow us to improve patient outcomes. On behalf of the College I would like to extend my sincere thanks for their foresight and commitment to this important field of medical endeavour.

We can and must do more. To ensure the future of surgical research we must continue to invest and enhance our ability to attract great research. I hope the following pages demonstrate our commitment to medical innovation and the transformative effect our work has had over the past two years. Ultimately, we all benefit from the discoveries and methods in this report, and I hope we can bring fresh hope to those patients who await the breakthroughs that come from incremental steps.

Introduction from the Chairman

The Research Committee has an important role in supporting the research and development activities of the College. Recently, there has been a reorganisation of two committees, the Research Strategy Committee (chaired by Professor Bob Steele, who has recently stepped down from Council) and the Research Allocation Committee (chaired by me). These two committees have now been merged into one, the Research Committee, which I chair. This reorganisation will provide a means of interacting with College Council and still delivering the assessment and adjudication of grant awards. The Ophthalmology sub-committee and Lorna Smith Charitable Trust will remain as 'offshoots' of the Research Committee.

The committee has been very busy and in the past few weeks almost £500,000 of funding has been awarded to members and fellows to support a very wide range of research projects. The committee is keen to have a more prominent outward-facing image on the revised College website so that we can interact better with the public and, in particular, potential donors and benefactors who may wish to support surgically relevant research. With this in mind we continue to interact with the development team to explore possible ways to improve our income and, therefore, the ability to support research.

Professor Stephen J Wigmore,
Chairman, Research Committee, RCSEd

For further information about the RCSEd's Research Programme and how to support it, please contact the Development Office, m.stitt@rcsed.ac.uk / 0131 527 1591

2014–16 RESEARCH REPORT IN NUMBERS

27 publications have come about through research projects funded by RCSEd

15 presentations have resulted from research projects in the Report

5 prizes have been won at international conferences

£1,042,860 is the total amount of funding in the Research Report

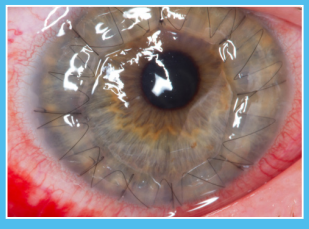
13 new international collaborations have been set up

4 new bodies have provided further funding

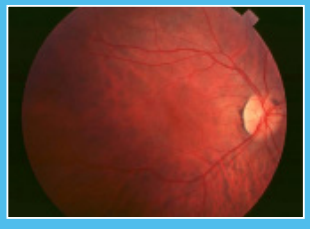
4 prizes have been won by researchers at conferences based on their projects

Research has been published in the most renowned journals in the world: *Nature*, *Proceedings of the National Academy of Science of the USA* and *The Lancet*

Notable projects

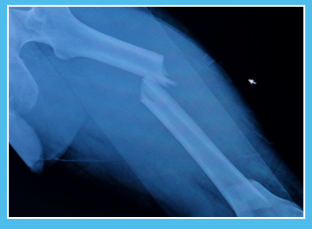


Professor Robert MacLaren
(University of Oxford)
works on photoreceptor transplantation to cure retinitis pigmentosa



Professor John V Forrester
(University of Aberdeen)
is researching corneal transplantation to overcome blindness

Parwez Hossain
(University of Southampton)
is studying ways to treat bacterial keratitis caused by the *Pseudomonas aeruginosa* bacterium



Professor Hamish Simpson
(The University of Edinburgh)
is researching how to stop non-union of fractures

Research Committee Members

CHAIRMAN

Professor Stephen Wigmore
Professor of Transplantation Surgery,
University of Edinburgh, Department of Clinical and Surgical Sciences, Royal Infirmary Edinburgh

MEMBERS

Professor Simon Paterson-Brown
Consultant General and Upper Gastrointestinal Surgeon,
Department of Surgery, Royal Infirmary of Edinburgh

Professor John H Dark
Professor of Cardiothoracic Surgery,
University of Newcastle, The Freeman Hospital

Mr Robert Jeffrey
Retired Cardiothoracic Surgeon, Aberdeen

Professor James P McDonald
Retired Dental Surgeon, Previous Dental Dean

Professor Hamish R Simpson
Professor of Orthopaedic Surgery and
Head of Department of Orthopaedic Surgery,
University of Edinburgh, Royal Infirmary of Edinburgh

Professor Michael Steel
Emeritus Professor of Medical Science and Honorary Consultant in Clinical Genetics for Tayside Health Board

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

Professor Paul Shiels
Professor of Cellular Gerontology, University of Glasgow

Dr Farhat Din
Senior Lecturer, Honorary Consultant Colorectal Surgeon,
University of Edinburgh

Mr Richard Montgomery
Honorary Treasurer,
Royal College of Surgeons of Edinburgh

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

Ophthalmology Sub-Committee

CHAIRMAN

Professor Stephen Wigmore
Professor of Transplantation Surgery, University of Edinburgh, Department of Clinical and Surgical Sciences, Royal Infirmary Edinburgh

MEMBERS

Dr David G Charteris
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

Dr Brian W Fleck
Consultant Ophthalmologist,
Princess Alexandra Eye Pavilion, Edinburgh

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

Mr Gordon Dutton
Consultant Ophthalmologist

Mr Richard Hellewell
Chief Executive, Royal Blind and
Scottish War Blinded, Edinburgh

Mr Richard Montgomery
Honorary Treasurer,
Royal College of Surgeons of Edinburgh

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

Donors to the RCSEd Research Fellowships and Grants

Mr Iain Fraser

Mr John Steyn and Family

The Cutner Memorial Bequest Fund

The Maurice Wohl Foundation

The Robertson Trust

Alastair F Jamieson

Royal Blind

Medical Research Council

The Lorna Smith Charitable Trust Research Fellowship

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

Fellowship Awards

THE ROBERTSON TRUST RESEARCH FELLOWSHIP

Mr Peter Young, Specialty Trainee Orthopaedics, University of Glasgow (£50,000)

"Effect of nanoscale surface topography on osteoclast differentiation and activity in orthopaedic materials"

Orthopaedic, spinal and dental surgery are dominated by the need to promote implant bonding to bone, especially joint replacements, which rank among the most successful healthcare interventions to improve quality of life. Prevalence of joint replacement is ever-increasing, with the prevalence of revision surgery for expired implants also climbing. Furthermore, osteoporosis is a major healthcare burden and, though medical intervention has been demonstrated to reduce risk of fracture, these drugs are not without side effects.

Precision engineering of surfaces with 'nanoscale' patterns (such as pits, which are much smaller than a human cell) on materials is cutting-edge and shows great promise. Recently, we have demonstrated the use of 'nanopatterns' to affect the behaviour of human stem cells.

In this project, we aim to establish the effect of nanoscale surfaces on bone-dissolving cells (osteoclasts) using unique cultures of human stem cells on orthopaedic materials such as titanium and plastics. We aim to identify features that may discourage bone turnover. These features will be potential targets for: (i) the design of orthopaedic implant surfaces to enhance implant lifespan; (ii) osteoporosis treatment.

THE ALASTAIR F JAMIESON FELLOWSHIP IN GENERAL SURGERY

Dr Amir Awwad, Clinical Radiology Specialist Registrar, University of Nottingham (£50,000)

"Four-dimensional flow magnetic resonance angiography of abdominal aortic aneurysms: a pilot study for growth and survival predictors"

The abdominal aorta is the largest blood vessel in the abdomen. If the vessel wall becomes weak, the vessel enlarges and the wall becomes thinner, thereby forming a balloon-like expansion called an 'aneurysm'. Such wall weakening carries the risk of rupture, leading to acute, significant blood loss and, usually, immediate death. To prevent this scenario, UK surgical practice offers aneurysm repair if the aneurysm has reached a presumed critical size: 5.5 cm. The repair is an open surgery or a keyhole approach (endovascular aortic repair).

However, the progression and outcome of aneurysms vary between individuals; some can become quite large (eg 10 cm) without rupturing and some may rupture early in the disease process. If we had better ways of predicting which aneurysms would rupture, we could target repair in those at high risk and avoid the risk and expense in those at low risk.

This study aims to expedite such prediction using novel non-invasive imaging to study the mechanical properties of the aneurysm wall to see if we can identify those at highest and lowest risk of rupture. We will investigate aneurysm growth with a new imaging tool: four-dimensional magnetic resonance angiography. This modality uses a series of short scans to study real-time images of an aneurysm that show relative blood pressure, flow and wall-strength properties to help identify signs of aneurysm growth and rupture risk.

THE MAURICE WOHL RESEARCH FELLOWSHIP IN SURGERY/DENTAL SURGERY

Iestyn Shapey, Specialist Trainee in General and Transplantation Surgery, University of Manchester (£50,000)

"Circulating unmethylated DNA and beta-cell death as a biomarker of graft dysfunction in pancreas and islet transplantation"

There is a shortage of high-quality organ donors appropriate for pancreas transplantation. In 2015, only 35% of pancreases offered for transplantation were transplanted. This shortage is coupled with the absence of a robust tool for objective assessment of their suitability for transplantation. The aim of this project is to identify a test that can accurately demonstrate that pancreas cells are alive and functioning, and hence the suitability for pancreas transplantation. The test could also be used to monitor transplant recipients for signs of worsening performance and loss of the transplanted pancreas.

THE JOINT RCSED/CUTNER FELLOWSHIP IN ORTHOPAEDICS

Jerry Tsang, Specialty Registrar in Trauma and Orthopaedics, University of Edinburgh (£50,000)

"An investigation into the effect physical modalities have on antibiotic efficacy in an *in vitro Staphylococcus aureus* biofilm model"

Professor Dame Sally Davies (Chief Medical Officer for England) has stated: "Antimicrobial resistance poses a catastrophic threat…routine operations like hip replacements or organ transplants could be deadly because of the risk of infection."

Staphylococcus aureus is one of the leading causes of infections from surgical implants and threatens to halt routine elective implant surgery. One strategy to overcome this problem is to look beyond 'traditional' antibiotics. Physical modalities, such as ultrasound and laser therapy, have been explored incompletely, but preliminary work has shown potential benefit. This research represents the initial phase of work to identify a novel method for the treatment of resistant staphylococci in the context of prosthetic joint infections.

THE LORNA SMITH CHARITABLE TRUST RESEARCH FELLOWSHIP

Tariq Farrah, Specialist Trainee in Renal Medicine/General Internal Medicine, University of Edinburgh (£55,526)

"MicroRNA Biomarkers in ANCA vasculitis: a prospective cohort study"

This study will prospectively follow patients with vasculitis (inflammation of blood vessels) and determine the relationship between a novel biomarker in blood and the natural history of the disease. Vasculitis is difficult to diagnose, and has a high prevalence of relapse and mortality. A new biomarker is needed urgently to guide treatment decisions.

We are studying a new small protein (microRNA) as a potential biomarker. This study will help us understand the relationship between microRNA and vasculitic activity. It could also lead to a clinical trial investigating if biomarkers reduce the risk of disease relapse compared with current methods.

MRC/RCSED CLINICAL RESEARCH TRAINING FELLOWSHIP

Mr Thomas Kurien, Academic Clinical Fellow ST5 Trauma and Orthopaedics, University of Nottingham (£114,337)

"Bone-marrow lesions and the central and peripheral drivers of knee osteoarthritis pain: a pre- and post-total knee replacement study"

Osteoarthritis (OA) is the most common form of arthritis worldwide. Pain is the predominant symptom, yet the central and peripheral drivers of pain are unclear. Total knee replacement (TKR) is the most effective surgical treatment for severe knee OA but, despite its success, ≤20% of patients have ongoing pain postoperatively. The cause of this pain is not known and treatment remains unsatisfactory.

Magnetic resonance imaging has demonstrated bone-marrow lesions (BMLs) within the subchondral bone in knee OA which correlate with pain. My research question is: "Do BMLs contribute to pain after TKR and what are the mechanisms involved?"

This novel research will lead to a more individualised or stratified medical approach for the treatment of patients suffering from pain due to knee OA. We aim to predict who will be the 80% of patients that benefit from TKR in the treatment of knee OA.

Travelling Fellowship Awards

CUTNER TRAVELLING FELLOWSHIP IN ORTHOPAEDICS

Mr Peter Domos, ST8 Orthopaedics, Peterborough City Hospital
"Visit to Centre Orthopédique Santy (Lyon, France)"
(£3,000)

THE JOHN STEYN TRAVELLING FELLOWSHIP IN UROLOGY

Dr Pankaj, Senior Resident, Department of Urology, Chandigarh
"Observership in Department of Urology, University Hospitals of North Midlands, Stoke on Trent"
(£900)

THE SIR JAMES FRASER TRAVELLING FELLOWSHIP IN GENERAL SURGERY

Miss Beatrix Elsberger, Clinical Lecturer in Surgical Oncology MD Anderson Cancer Center (Houston, TX, USA)
"Observation of training in surgical oncology at (especially) breast cancer care; opportunity to compare the British and American systems; observation of new imaging technology in surgical practice; establishing collaboration for future translational research projects on breast cancer"
(£1,500)

Ophthalmology Awards

FUNDED BY ROYAL BLIND

SMALL PROJECT GRANTS

Harminder Dua, Academic Section of Ophthalmology, University of Nottingham
"Evaluation of antimicrobial peptide synergism against ocular surface pathogens"
(£49,151

MAJOR PROJECT GRANTS

John Forrester, School of Medicine and Dentistry, University of Aberdeen
"In-depth studies of innate and adaptive immunity in *in vitro* analyses of crosslinked recombinant human collagen hydrogels and dendritic cells in corneal regeneration for pre-clinical applications"
(£49,960)

Umiya Agraval, Tennent Institute of Ophthalmology, Gartnavel General Hospital, Glasgow
"Patient-reported outcomes: are we performing cataract surgery too early?"
(£12,500)

Robert MacLaren, Nuffield Laboratory of Ophthalmology, University of Oxford
"Optimising gene therapy for dominant retinitis pigmentosa"
(£50,000)

Mandeep Sagoo, UCL Institute of Ophthalmology
"Identification of new mechanisms and targets in retinoblastoma"
(£49,985)
It was agreed that the Chair would write to Dr Sagoo asking for a new layman summary for Richard Hellewell because the layman summary in his application form was too technical.

Parwez Hossain, Academic Unit of Clinical Experimental Sciences, Faculty of Medicine, University of Southampton
"Resolving the mechanisms of host inflammasome activation in microbial keratitis"
(£49,993)

Shareen Forbes, Endocrinology Unit, University of Edinburgh
"Impact of insulin pump therapy and islet transplantation on progression of diabetic retinopathy in type-1 diabetes"
(£50,000)

Robert MacLaren, Nuffield Laboratory of Ophthalmology, University of Oxford
"Optimising gene therapy to improve patient safety"
(£49,989)

John Forrester, School of Medical Sciences and Nutrition, University of Aberdeen
"*In vitro* responses of dendritic cells to crosslinked recombinant human collagen hydrogels in corneal regeneration for pre-clinical applications"
(£49,971)

Andrew Tatham, Princess Alexander Eye Pavilion (NHS Lothian), Edinburgh
"The Scottish Glaucoma Biobank: developing a national resource for the study of disease mechanisms, risk markers for blindness and novel drug discovery in glaucoma"
(£49,768)

King James IV Professorship Awards

Mr Justin Durham, Diplomate of American Board of Orofacial Pain, Senior Lecturer in Oral Surgery and Orofacial Pain, School of Dental Sciences, Newcastle
"Conjuring up clarity in the 'black art' of diagnosis and management of chronic orofacial pain"
(£500)

Professor Kalu Ogbureke, Professor and Chair of Department of Diagnostic and Biomedical Sciences, the University of Texas
"The SIBLING family of proteins and their cognate matrix metalloproteinases in oral cancer"
(£500)

Mr Robert Paton, Consultant Orthopaedic Surgeon, Visiting Professor, School of Medicine and Dentistry, University of Central Lancashire, Honorary Senior Lecturer, University of Manchester
"Clinical and sonographic screening in pathological developmental dysplasia of the hip"
(£500)

Small Research Grants

Mr Ernest Azzopardi, Swansea University
"Amylase activity in localised infection"
(£9,000)

Mr Jason Wong, University of Manchester
"The role of macrophages in vascularisation and tissue formation in an AV loop model of tissue generation"
(£10,000)

Mr Jaimin Bhatt, University Health Network, Canada
"An old landscape through new eyes: aminoglycosides as a potential therapy for renal cancer"
(£7,500)

Mr Jay Nath, University of Birmingham
"Metabolic characterisation of machine-perfused kidneys"
(£9,564)

Mr Vinnie During, Urology Research Registrar, University of Birmingham
"An investigation of the immune microenvironment of bladder cancer and its clinical application"
(£10,000)

Mr Adam Frampton, Honorary Clinical Lecturer in General Surgery, Imperial College London
"MicroRNA markers in bile for detecting and stratifying pancreatic cancer"
(£9,945)

Mr Gurdeep Mannu, Academic Clinical Fellow, University of Oxford
"A case–control study to investigate the lifestyle and biological risk factors for developing ductal carcinoma *in situ* of the breast and its progression to invasive breast cancer"
(£2,700)

Mr Iain Murray, ECAT Clinical Lecturer, ST3, University of Edinburgh
"Therapeutic targeting of myofibroblasts in skeletal muscle fibrosis"
(£9,961)

Miss Emma Scott, Clinical Research Fellow University of Edinburgh
"Contrast-enhanced magnetic resonance imaging as a diagnostic tool for chronic allograft damage following renal transplantation"
(£10,000)

Mr Andrew Sutherland, Specialist Registrar, University of Edinburgh
"Improving graft function in transplantation with normothermic regional perfusion: a pilot study to investigate the underlying mechanism"
(£9,772)

Mr Gerald J McKenna, Consultant in Restorative Dentistry and Senior Lecturer, Queen's University, Belfast
"Developing an evidence-based decision-making tool for tooth replacements for older patients"
(£9,683)

Mr Michael Ramage, Specialty Registrar in General Surgery, University of Edinburgh
"Mechanisms of muscle loss in cancer cachexia"
(£9,480)

Mr Michail Feretis, Hepatobiliary Surgical Research Fellow, Addenbrooke's Hospital, Cambridge
"Developing clinically useful measures of CXCR4-mediated tumour immune privilege"
(£9,500)

Mr Michael McLean, Specialty Registrar in Trauma & Orthopaedics, University of Glasgow
"Influence of topical tranexamic acid on the cellular and matrix integrity of human cartilage, ligament and tendon: *in vitro* study"
(£9,300)

Dr Sarah Waring, Academic Clinical Lecturer/ Honorary Specialty Registrar in Oral & Maxillofacial Pathology, University of Birmingham
"Prognostic models of oral cancer outcome based on morphological invasiveness"
(£8,748)

Mr Adam Frampton, Honorary Clinical Lecturer in General Surgery, Imperial College London
"The microRNA processing endoribonuclease Dicer has altered expression in pancreatic ductal adenocarcinoma and prognostic implications"
(£9,712)

Mr Jason Wong, Academic Consultant Plastic Surgeon, University of Manchester
"*In vivo* reprogramming of transplantable tissue using the mouse AV loop"
(£9,438)

Dr Nick Kalson, NIHR Academic Clinical Fellow in Orthopaedics, Newcastle University
"Investigation of signalling pathways driving tissue fibrosis after total knee replacement"
(£9,997)

Mr Carlo Ceresa, Clinical Research Fellow in Transplantation, University of Oxford
"Effects of normothermic machine perfusion on human steatotic livers utilised for transplantation"
(£9,550)

Dr Rachel Guest, Clinical Lecturer and Honorary Specialty Registrar in General Surgery, University of Edinburgh
"An unbiased screen for novel molecular biomarkers of the progression of primary sclerosing cholangitis to cholangiocarcinoma"
(£9,000)

Mr Iestyn Shapey, Specialist Trainee in General and Transplantation Surgery, University of Manchester
"Circulating unmethylated DNA and beta-cell death as a biomarker of graft dysfunction in pancreas and islet transplantation"
(£10,000)

Student Bursaries

Mr Cameron Alexander, Nuffield Department of Surgical Sciences, University of Oxford
"Recurrent urological stone disease: can it be predicted and what are the implications of repeated ionising radiation exposure?"
(£1,000)

Miss Sadhia Khan, ENT Department, Salford Royal Hospital
"Regrowth of surgically treated vestibular schwannomas in type-2 neurofibromatosis"
(£900)

Miss Priyadarssini Karunakaran, Temple Street Children's University Hospital, Dublin
"Volumetric and craniometric changes in posterior cranial vault distraction for syndromic craniosynostosis"
(£1,200)

Miss Teo Yong Al Roxanne, University of Cambridge
"Inhibition of humoral alloimmunity by transfer of third-party regulatory T cells with direct allospecificality for host MHC alloantigen"
(£1,000)

Mr Mark Twoon, Plastics and Reconstructive Surgery Unit, University of Aberdeen
"Lateral mammoplasty instructional video"
(£900)

Mr Shaun Evans, NIHR Surgical Reconstruction and Microbiology Research Centre, University of Birmingham
"Principal component analysis of the serum microRNA response to traumatic brain injury"
(£1,500)

Miss Mollika Chakravorty, Guy's and Saint Thomas' NHS Foundation Trust
"An economic and clinical comparison of the outcomes following robotic versus laparoscopic nephrectomy"
(£750)

Mr Tom Drake, Multidisciplinary Cardiovascular Research Centre, University of Leeds
"The role of store-operated calcium signalling in colorectal cancer metastasis"
(£780)

Mr Moiz Hazeeb, Academic Centre for Reconstructive Science, King's College London
"Assessing the accuracy of low-cost fused deposition modelling in three-dimensional printing of maxillofacial prosthetics"
(£900)

Mr Matthew Arnold, Department of Orthopaedics, Edinburgh Royal Infirmary
"Effect of varying amounts of decalcification and de-collagenisation on bone strength and toughness"
(£1,500)

Mr David Williams, School of Marine Sciences and Technology, Newcastle University
"NucB and the prevention of microbial formation in orthopaedic ankle fracture fixation"
(£1,500)

Syme Medal Awards

Mr Aman Chandra, Vitreoretinal Fellow, Royal Victoria Eye and Ear Hospital, Australia, and Moorfields Eye Hospital, London
"The genetic associations of rhegmatogenous retinal detachment and ectopia lentis"

Mr Grant Stewart, Clinical Senior Lecturer and Honorary Consultant in Urological Surgery, University of Edinburgh and NHS Lothian
"Prognostic and predictive biomarker development in renal cell cancer"

Miss Rachel Guest, Academic Clinical Lecturer in General Surgery and Honorary Specialty Training Registrar, University of Edinburgh
"Defining the functional role of Notch signalling in Intrahepatic Cholangiocarcinoma"

Mr Mark Hughes, Clinical Senior Lecturer and Honorary Consultant in Urological Surgery, University of Edinburgh
"Prognostic and predictive biomarker development in renal cell cancer"

Mr John Robert O'Neill, Specialty Registrar in General Surgery, South East Scotland
"Strategies to identify novel therapeutic targets for oesophageal adenocarcinoma"

Fellowship Reports

Fellowship Report

Predictive modelling in organ-confined 'low risk' muscle-invasive bladder cancer

Miss Jennifer Jones
Urology, Robertson Trust Research Fellowship

1 August 2013 to 1 August 2014

LAY SUMMARY

Most people with bladder cancer have tumours that grow out from the bladder lining, which can be removed with keyhole surgery. However, some tumours grow into the bladder muscle, necessitating removal of the entire bladder along with the tumour at surgery. If a tumour grows into the bladder muscle but does not extend beyond it, around three-quarters of people will be cured by bladder-removal surgery alone. However, identification of the one-quarter of people who may benefit from additional chemotherapy in this group is difficult. Given that systemic chemotherapy has extensive side-effects, we continue to look for ways to ensure we are not 'over-treating' most patients, while ensuring that we are not 'under-treating' those at higher risk of cancer-related death.

This study looked at these specific types of bladder cancers and examined the genes turned 'on' and 'off' between people who have lived and died (the three-quarters versus the one-quarter). We found that markers that can predict cancer-related death appear to be related to the immune system and to the spread of tumours into the local lymphatic system of the bladder (which is usually associated with more advanced disease). This biomarker profiling may enable identification of these high-risk patients, and could allow examination of alternative treatments.

GRANT REPORT

(A) CLINICAL AND SCIENTIFIC SIGNIFICANCE OF ADVANCES MADE

1. PROFILING OF BIOMARKERS THAT CAN PREDICT ORGAN-CONFINED, MUSCLE-INVASIVE BLADDER CANCER

Background

The worldwide annual incidence of bladder cancer is >400,000 cases per year, with ≈100,000 of these cases being classed as muscle-invasive bladder cancer (MIBC). MIBC requires radical therapeutic intervention to achieve disease control (radical cystectomy ± systemic cisplatin-based chemotherapies).[1] Implementation of chemotherapies is reserved for 'high-risk' subpopulations, though international guidelines state that there are no clear indicators of high-risk status other than standard staging assessment alone.[1] In the population of pT3/4 disease (in which extravesical extension of tumours is microscopic or macroscopic), there is a clear risk of metastatic disease. In studies of isolated radical cystectomy of advanced disease, ≤25% of patients had positive lymph nodes at initial surgery[2] but, in the cohort of patients with organ-confined MIBC (pT2a+b), the prevalence of lymph node-positive status at cystectomy was low.[2] However, 5-year mortality approached 50% despite patients

Predictive modelling in organ-confined 'low risk' muscle-invasive bladder cancer

having undergone supposedly curative radical intervention. Use of systemic chemotherapy in this cohort is advocated for those at high-risk of cancer-specific mortality, but ≈75% of these patients are classified as 'low risk' and addition of chemotherapy would expose around two-thirds of this cohort to unnecessary morbidity.[1] There is evidence that survival may be linked to the molecular subtype of bladder cancer. However, the findings remain inconsistent, with only certain 'luminal' tumours suggesting a good prognosis and most other subtypes linked with intermediate survival.[3, 4] This study, therefore, sought to examine the differences, at a genomic level, of patients who died of pT2a/b MIBC compared with survivors. Then, we examined the different pathways involved and formulated a predictive model of survival of these patients and validated our findings at genomic and proteomic levels.

Results

A meta-analysis of publicly available gene-expression datasets was undertaken (GSE 38294, 48075, 48274) for 515 samples of bladder cancer of all grades and stages, of which 92 samples were pT2(a&b) disease. An initial test batch of 42 samples from a single dataset was used as a training dataset and 54 samples combined into a second validation cohort. All data underwent probe filtration, normalisation, batch correction, and cross-platform normalisation to allow appropriate comparisons.

Differential expression of genes was determined by rank product, including relative upregulation and downregulation of expression. Non-functioning genes were excluded from further modelling. Then, 431 genes and five prognostic clinical domains (grade, stage, lymph-node status, sex, age) were incorporated into a random-Forest model and an optimum three-gene classifier (cluster of differentiation (CD)96, chondroitin polymerizing factor (CHPF), C-C motif chemokine receptor (CCR)7) built with classification and regression tree (CART) modelling (Figure 1).

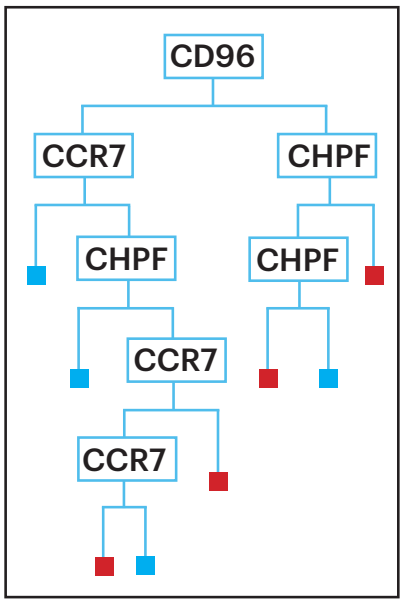


Figure 1. Three-gene predictive classifier

Within the training dataset, this strategy produced a positive-predictive value (PPV) of 100% and a negative predictive value (NPV) of 100%. When the classifier was applied to the validation dataset, the PPV was 84.6% (95% confidence interval (CI), 53.6–97.3) and the NPV was 81.0% (65.4–90.6). A second validation dataset of next-generation sequencing data from 27 samples within the The Cancer Genome Atlas produced a PPV of 92.3% (95% CI, 62.1–99.6) and NPV of 85.7% (56.6–97.5). Application to all MIBC samples saw a reduction in predictive power of the model with an overall PPV of 60.0% (95% CI, 44.4–73.9) and NPV of 61.5% (49.8–72.1). Sub-stage analyses revealed that the gene classifier was accurate for pT2a–pT3a tumours but not so for pT3b/4 tumours. Pathway analyses revealed the predominant pathways with differential expression to include wnt signalling, cell–cell adhesion, PI3K–Akt signalling and chemokine signalling, though

Predictive modelling in organ-confined 'low risk' muscle-invasive bladder cancer

no specific pathway in isolation was predictive. There was evidence of some pathways being enriched due to particular pathological variants of tumours (upregulated hippo signalling due to samples being predominantly squamous tumours). We are examining the impact of these genes at the proteomic level.

Discussion

The three-gene classifier defined in this work is based on expression of the stromal markers CD96, CHPF and CCR7. CD96 is a type-1 membrane protein and involved in adhesion of activated T-cells and natural killer cells during the late-phase immune response and has been investigated in myelo-proliferative leukaemias.[5] CHPF is a glycosaminoglycan and may have a role in protecting cells from oxidative stress; one study correlated proteomic expression to increasing cancer stage.[6] CCR7 is a member of the g protein-coupled receptor family and associated with activation of B-cells, T-cells and lymphatic vessels.[7] Studies have investigated CCR7 as a marker of progression in MIBC[8] whereas expression of C96 or CHPF in bladder cancer is unreported.

There has been increasing interest in molecular subtyping of MIBC. Results have revealed similar subsets to those identified in breast cancer (basal, luminal, claudin-low, normal breast-like).[3, 4] Those studies have reported better survival outcomes in luminal-type tumours, but none of the studies have reported a specific 'poor prognosis' group (though the squamous subtype has been linked to higher staging and positive lymph-node metastasis in cystectomy studies).[9, 10]

This study incorporated all molecular subtypes of tumours to identify a predictive model. We found that the most predictive differences in gene expression were for stromal features, irrespective of subtyping of specific tumours. The concept of an immune role in bladder cancer is accepted. Patients at high risk of non-muscle-invasive bladder cancer (NMIBC) have Bacillus Calmette–Guerin injected directly into the bladder, which stimulates a non-specific immune response and improves recurrence- and progression-free survival.[11] However, this model identifies high expression of T-lymphocyte-related genes in survival of patients with MIBC, which requires further investigation (currently ongoing with proteomic studies).

Our examination revealed that clinical variables had limited predictive power, though overt lymph-node metastasis at cystectomy was the most reliable predictor of survival. The finding of lymph-related factors as predictors supports the clinical data, but we continue to examine lymphovascular invasion and density of lymphatic vessels as more accurate predictors.

Several studies have examined the relationship between activated stromal components and survival. However, use of such predictive tools within this project remains unexplored and allows a specific framework for further studies.[12, 13] The initial aim of this predictive model was to aid risk stratification at the initial diagnosis and staging, allowing appropriate selection of patients for adjunctive chemotherapy. However, the results may suggest a group in whom alternative immunotherapy may be appropriate. Findings of a prognostic role for T-lymphocytes have been identified in other immune studies, and work continues to determine the exact nature of this

Predictive modelling in organ-confined 'low risk' muscle-invasive bladder cancer

role. New immunotherapies using programmed-cell death ligand-1 pathways are showing promise in management of advanced bladder cancers.[14–17] The change in predictive power of the model with varying pathological stage suggests that once stromal defences have been breached, other factors (eg epithelial–mesenchymal transformation (EMT)) have a greater role than the remaining stroma. We are continuing to examine this change in advanced disease using a similar predictive framework of gene-expression analyses.

2. 'CLAUDIN-LOW' BLADDER CANCER AND ITS RELATIONSHIP WITH THE HIPPO SIGNALLING PATHWAY

Introduction

Urothelial cancer is a heterogeneous disease associated with a vast range of outcomes with respect to the recurrence and progression of cancer as well as disease-specific survival. Gene-expression classifiers have been used to identify differences in expression profiles between low- and high-grade lesions within NMIBC and MIBC, with profiles developed to predict lymph-node status, progression and tumour stage. Subdivision of high-grade lesions into intrinsic subtyping has seen the emergence of two main tumour groups: basal-like and luminal.[3] At the gene-expression level, the tumours seem to share biological similarities with breast cancer and include the claudin-low subgroup of basal-like tumours.[18]

Hippo signalling is a cellular pathway that controls organ size via regulation of cell proliferation, apoptosis, and self-renewal of stem cells.[19, 20] It involves a kinase cascade that phosphorylates and inactivates YAP and TAZ (major effectors of the hippo pathway). In a dephosphorylated state, YAP and TAZ enter the nucleus and interact with transcription factors to induce gene expression of factors involved with cell proliferation and inhibition of apoptosis. Uncontrolled proliferation of cells is a key process in tumour formation and insensitivity to apoptosis is vital to tumour propagation, so the potential role of the hippo signalling pathway merits investigation.[20] Furthermore, epithelial tumours in particular have a higher degree of organisation and architecture of cells then other tissues, which is associated with the hippo function of maintaining cell polarity and cell–cell adhesion. Studies on the hippo pathway in breast tumours have shown a link to the claudin-low subset of basal-like tumours, with an association of over-expression of YAP/hippo signalling in claudin-low tumours.[21] In this meta-analysis we investigated if, at a gene-expression level, we could identify a correlation between urothelial tumours and classifiers of hippo signalling, and whether these tumours are aligned within the claudin-low subsets of urothelial tumours.

Results

Claudin-low tumours were identified by their high expression of CD44 and low expression of all other basal and luminal markers (these tumours are often referred to as 'triple-negative' tumours) using a 28-gene basal/luminal classifier. Overall, 22 tumours (from a series of 359 tumours) were identified as claudin-low (6.1%). These tumours showed enrichment of EMT markers, upregulation of a conserved YAP 56-gene signature, and

Predictive modelling in organ-confined 'low risk' muscle-invasive bladder cancer

high expression of a 20-gene hippo signalling signature within the bladder-cancer dataset. Using a second 725-gene signature from a previous breast-cancer study, claudin-low samples were confirmed as an independent subset. Using the breast signature, basal samples were divided into 'basal' and 'normal breast-like' although, in the bladder classifier, the normal breast-like samples were amalgamated into a single subset. In addition to claudin-low tumours, we found greater enrichment of YAP/hippo signalling in bladder tumours with squamous pathology, which aligned with the normal breast-like subset of breast tumours. This group was distinct from other basal types of tumours due to lower expression of claudins and cadherins within the normal-breast-type subset compared with other basal tumours.

Discussion

With advancing understanding of molecular subtyping of urothelial tumours there is an urge to ascertain if these subtypes carry clinical impact. Studies suggest that luminal tumours (which bear the greatest resemblance to a normal epithelial structure) have the best associated prognosis and basal tumours, with their association with squamous properties, have a worse prognosis.[4] Claudin-low tumours are enriched with mesenchymal and stem-cell markers. They may relate to sarcomatoid-like features, which are associated with high metastatic potential in upper- and lower-tract urothelial cancers (though the exact nature of claudin-low tumours remains poorly understood).[22]

This is the first study to link claudin-low bladder tumours to enrichment of the hippo signalling pathway. Claudin-low tumours have a similar profile to the basal-tumour subset in YAP/hippo gene signatures but remain distinct due to lower expression of E- and P-cadherin. The clinical significance of this finding is not known, and we are beginning to examine the hippo pathway in bladder cancer cell lines. With regard to breast cancer, the claudin-low/triple-negative tumours represent a particularly problematic cohort because they are resistant to standard hormonal manipulation.[23] Studies have suggested these tumours could be subdivided further into 'immune rich' and 'immune poor' to direct potential immunotherapies, but those studies were small and unvalidated.[23] Of further concern for this subset of tumours is that they appear to be resistant to cisplatin-based chemotherapy, which is the primary choice of chemotherapy for bladder tumours.[24] However, identification of the claudin-low population of bladder tumours remains a challenge (particularly at a clinical level). Identifying features that may help us isolate these tumours for further investigation is continuing.

Predictive modelling in organ-confined 'low risk' muscle-invasive bladder cancer

(B) PROBLEMS ENCOUNTERED AND STEPS TAKEN TO OVERCOME THEM

Biomarker profile

The initial meta-analysis was suboptimal due to exclusion of pathologies of variant tumours from the biomarker model, in which the hypothesis was that the molecular pathogenesis of different tumour types would act as a confounding factor. This approach limited sample sizes and reproducibility of the model at validation. An improved model was formulated upon inclusion of all variant pathological types, with a PPV of 84.6% and NPV of 81.0% upon validation.

Validation of samples

We did not have a sufficient number of samples with adequate clinical information within our bank to complete a second proteomic validation. We have developed links to urology academic research centres in the USA who will provide tissue and clinical details for validation.

Urine studies

A further aspect of the original project was to collect urine samples for a validation study on use of mini-chromosome maintenance protein-2 as a proliferation marker for identification of NMIBC within NHS Lothian. Tissue collection was undertaken after approval of the relevant ethics committees and Research and Development office of the National Health Service (NHS). However, implementation of collection in NHS Lothian was poor and led to early discontinuation of the initial validation after 50 patients. However, the clinical infrastructure within NHS Fife with commencement of a new urology diagnostic suite has led to the study being transferred to that site.

(C) COLLABORATIONS ESTABLISHED

- Examination of claudin-low bladder tumours and the relationship to hippo pathway signalling at the University of St Andrews, St Andrews, Scotland
- Examination of application of tissue phenomics in 'low-risk' muscle invasive bladder cancer – Dinney/McConkey research team, MD Anderson Cancer Center (Houston, TX, USA)
- Validation of current biomarker project – Clarke research team, Vanderbilt University (Nashville, TN, USA)

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

Jones J, Turnbull A, Harrison D.
'Predictive modelling of pT2 muscle-invasive bladder cancer'
International Cancer Colloquia: Bladder Cancer
St Andrews, Scotland, February 2015

This work represents a contribution towards my current project, which culminated in the award of a doctorate in August 2015.

Further funding was awarded from the Melville Trust for the Care and Cure of Cancer for 2014/2015.

Predictive modelling in organ-confined 'low risk' muscle-invasive bladder cancer

(E) ACKNOWLEDGEMENTS

Supervisors

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Edinburgh Urological Cancer Group

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Bioinformatics

Dr Arran Turnbull

Tissue phenomics

Dr Peter Caie

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Fellowship Report

Correction of orthopaedic deformities in children

James Charles Beazley
Paediatric Orthopaedics, Beit Cure International Hospital, Blantyre, Malawi
Cutner Travelling Fellowship
14 August 2013 to 14 July 2014

LAY SUMMARY

In July 2014 I completed an 11-month fellowship at Beit Cure International Hospital (BCIH) in Blantyre, Malawi, for which I was very kindly supported by the Cutner Travelling Fellowship.

Malawi has a population of 16 million and is ranked among the 10 poorest countries in the world. BCIH is the only paediatric orthopaedic centre in Malawi and treats a diverse and complicated set of orthopaedic conditions using contemporary methods. I gained excellent clinical experience at BCIH which is transferable to UK practice. Owing to the diversity of the caseload, surgical procedures at BCIH were frequently not routine. Consequently, BCIH has developed a culture of detailed preoperative planning. Development of my preoperative planning skills at BCIH has provided me with the platform to advance the complexity of procedures I can undertake safely.

In addition to advancement of my clinical skills, the fellowship at BCIH enabled me to design and run a small randomised trial examining if the timing of dressing changes affects skin-graft survival after burn contracture release (BCR). This research will provide locally useful data, and has provided me with valuable experience in setting up and running a randomised trial in a resource-poor environment.

GRANT REPORT

(A) CLINICAL AND SCIENTIFIC SIGNIFICANCE OF ADVANCES MADE

Clinical advances

The mainstay of the workload was correction of club foot, tibial and femoral deformities, treatment of chronic osteomyolitis and its sequelae, and burn contracture release.

BCIH has five orthopaedic consultants. As the Fellow, I was under the supervision of John Cashman and Nicholas Lubega, who trained in the UK and Uganda, respectively. John Cashman has a particular interest in deformity correction with circular frames. The orthopaedic equipment in the surrounding district hospitals was very poor, but the equipment at BCIH was of a high standard. An abundance of donated Illizarov and Taylor special frames ensured that we could use contemporary methods to address the complicated deformities with which we were presented. This ensured that the skillset I developed over the year is transferable to UK practice.

Owing to the diversity and severity of caseload, the operative experience I gained at BCIH was excellent. Initially all procedures were supervised by John or Nicholas, with increasing independence as the fellowship progressed. I undertook >30 surgical club-foot corrections and 40 corrections of tibial or distal femoral

Correction of orthopaedic deformities in children

deformities. Twenty-five percent of the surgical workload at BCIH is BCR, which enabled me to develop a set of simple skills related to plastic surgery, including skin grafting and local flaps.

I spent a considerable amount of time initially with John Cashman and later independently planning procedures, often with tracing paper models (digitalised radiographs have yet to arrive in Malawi). Detailed procedural plans would be generated and taken into theatre. As an adjunct to preoperative planning, if deformity correction involved use of circular frames, all frames were prebuilt. This policy enabled me to 'get the miles in' on the frames without the time pressure of theatre. A good example of this approach was club-foot correction with frames.

A circular frame was used to treat a left-sided club foot. It had eight poly-axial hinges and drivers to provide correction of the multi-planar deformity. Building these frames initially was very time-consuming. Pre-building these frames greatly reduced theatre time and accelerated independent operating. Detailed preoperative planning (including pre-building frames) has enabled me to increase the complexity of procedures I can undertake safely and independently, which I will translate to my UK practice.

Research

Owing to difficulties with follow-up, it quickly became apparent meaningful research would have to be done prospectively. BCR constitutes ≤25% of the BCIH workload. Local audit data identified a need to investigate if the timing of change of the first dressing affects the outcome of full-thickness skin grafting. A literature review identified no published research on the subject. I designed a protocol, gained approval from the ethics committee and recruited 50% of the required patients for a small randomised trial. This research is ongoing and, though it will not provide a breakthrough in burns care, it will provide locally useful data. Also, it gave me valuable experience in setting up and running a randomised trial in a resource-poor environment. In addition, I was involved with data analyses of a national trauma audit examining the burden of trauma on regional hospitals in Malawi and in the follow-up of patients undergoing reconstruction of the medial patellofemoral ligament. These data were presented at the Malawi Surgical Association in May 2014 and the College of Surgeons of East, Central and Southern Africa conference in November 2013.

Teaching

I taught at undergraduate and postgraduate levels delivering lectures regularly to the fourth-year medical students at the College of Medicine in Blantyre. I acted as an examiner for the fourth-year objective structured clinical examinations and fifth-year final examinations. At the postgraduate level, I taught an AO non-surgical course in Mangochi, a course on club foot at BCIH, a Train the Trainers Basic Surgical Skills course and a Basc Surgical Skills Course in Kampala, Uganda.

Correction of orthopaedic deformities in children

(B) PROBLEMS ENCOUNTERED AND STEPS TAKEN TO OVERCOME THEM

Gaining ethics committee approval took time, but persistence paid off and the randomised trial was approved.

We encountered difficulties obtaining temporary employment permits, but found talking directly to the head of the permits division very helpful. My partner broke her radial head and neck and her first metatarsal within 8 weeks of each other. Fortunately, she was well placed to receive care at BCIH. In many respects the challenge of overcoming these difficulties (particularly for my partner) added to the experience gained in Malawi.

(C) COLLABORATIONS ESTABLISHED

Strong links established with BCIH. Research is ongoing.

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

Chokotho L, Mulwafu W, Jacobsen KH, et al. Assessing the burden of trauma in four district hospitals of Malawi: a retrospective medical record review. Malawi Surgical Association, Blantyre, 2014

Cashman J, Beazley J, Gardner R. Medial patellofemoral ligament reconstruction in children and adolescents – the CURE procedure. Description of technique and early results. College of Surgeons of East, Central and Southern Africa, Harare, 2013

(E) ACKNOWLEDGEMENTS

I thank BCIH for the opportunity to work as the Antonia Freeman Fellow and the Royal College of Surgeons of Edinburgh for their very generous award of the Cutner Travelling Fellowship.

Travelling Fellowship Reports

Travelling Fellowship Report

Cutner Travelling Fellowship

Graham Dall

Institute for Foot and Ankle Reconstruction, Mercy Medical Center, Baltimore, MD, USA

January 2014 to July 2014

LAY SUMMARY

The fellowship is renowned worldwide and attracted many visiting professors and fellows during my 6 months working with Dr Mark Myerson. I obtained a massive amount of operative experience (330 cases) in complex reconstruction of the foot and ankle. In particular, I gained experience in revision of ankle replacements that would have taken me many years to observe in the UK.

I worked on basic science and clinical research projects, and presented my findings at national meetings.

I attended the American Academy of Orthopaedic Surgeons in New Orleans, and exchanged ideas with leading foot-and-ankle surgeons.

Overall, it was a fantastic fellowship and I would recommend it to anyone with an appetite for working hard and honing their specialist interest in surgery of the foot and ankle.

GRANT REPORT

A) CLINICAL AND SCIENTIFIC SIGNIFICANCE OF ADVANCES MADE

Obtained expertise in correction of multi-planar deformities of the foot and ankle that would have taken years to accrue in the UK due to the super-specialisation and reputation of Dr Myerson in managing severe deformities.

(B) PROBLEMS ENCOUNTERED AND STEPS TAKEN TO OVERCOME THEM

Snowstorms, home sickness and sleep deprivation were overcome by new friendships and fantastic memories of Baltimore.

(C) COLLABORATIONS ESTABLISHED

Professor Maceria, Lew Schon, Rebecca Ceratto and Mark Myerson.

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

Presented our results of a case–control study looking at use of the Cotton osteotomy in flatfoot reconstruction at a meeting on surgery of the foot and ankle in Brighton, UK, in November 2014.

(E) ACKNOWLEDGEMENTS

Thanks to the Cutner Fellowship I have been fortunate to have a fantastic fellowship that will stand me in good stead during my consultant career.

Travelling Fellowship Report

Cutner Travelling Fellowship

Mr Alexander Aarvold
Consultant Paediatric Orthopaedic Surgeon at Southampton Children's Hospital
Travelled to British Columbia Children's Hospital, Vancouver, Canada
August 2014 to July 2015

INTRODUCTION

British Columbia Children's Hospital (BCCH), Vancouver, Canada, is the only children's hospital in British Columbia (BC) and the Yukon. BCCH serves >1 million children from a landmass >15-times the size of England. One patient had travelled for 2 days to his clinic appointment. Rare disorders and deformities are, therefore, routine in the clinics at BCCH and the experience of a 1-year fellowship has been fantastic. This period of training has, against a background of higher surgical training in Wessex, prepared me very well for my consultant post at Southampton Children's Hospital.

CLINICAL EXPERIENCE

The direct clinical training at BCCH is exceptional. Clinic templates are based on consultant-only practice, so there was ample time to discuss cases. Each clinical encounter could be used as a case-based discussion. I have 700 procedures in my logbook from this fellowship and I barely assisted a case all year. As soon as I had completed a procedure, I was expected to supervise a resident the next time. As proponents of the 'learning pyramid' will know, teaching is the most effective form of learning. This is a practice that I will employ in the National Health Service (NHS).

I gained competence in many procedures: limb-growing rods for osteogenesis imperfecta; single-event multi-level surgeries (SEMLS) for crouch gait; neuromuscular hip reconstructions; open reductions for late developmental dysplasia of the hip (DDH); pelvic osteotomies for bladder exstrophy; femoral osteotomies (varus, valgus, rotational, shortening); surgical dislocation and Dunn osteotomy for severe slipped upper femoral epiphysis (SUFE); patella-realignment strategies for congenital/habitual/adolescent dislocations' hemi-pelvis lengthening; femoral-neck lengthening 'Morscher' osteotomy; the 'super hip' procedure for proximal femoral focal deficiency; postero-medial releases for arthrogryposis; and circular frames for a wide spectrum of extraordinary deformities.

Throughout the year I rotated to work with Drs Kishore Mulpuri (hips and neuromuscular), Tony Cooper (deformity and frames), Christine Alvarez (feet) and Chris Reilly (sports and spines). This strategy provided me with broad-based experience in paediatric orthopaedics combined with extensive exposure to quarternary-level care.

MENTORSHIP

The mentorship system is experiencing resurgence in the UK but is well established in Canada. In Drs Mulpuri and Cooper I had exceptional mentors whose approaches I emulated. We discussed at length all aspects of practice, enriching the already exceptional clinical ▷

Cutner Travelling Fellowship

training. I was lucky to work with co-fellows Dr Sasha Carsen (now consultant paediatric orthopaedic surgeon at the Children's Hospital of Eastern Ontario in Ottawa, Canada) and Dr Lisa Phillips (now consultant paediatric orthopaedic surgeon at Alberta Children's Hospital in Calgary, Canada). We worked well as a team, combining in clinics and theatre to maximise our exposure, learning from each other and putting the world's medical politics to rights! These conversations will continue at conferences throughout our careers and (hopefully) as lifelong friends.

My first-hand experience of the Canadian healthcare system as a doctor (and also when my wife lacerated her hand flexor tendon within a few weeks of us arriving in Canada), plus exposure to the American system, provided a clear contrast to the NHS. There are attributes and deficiencies in all systems, but the undoubted strengths of the NHS have been reinforced to me.

RESEARCH EXPERIENCE

Research opportunities abound. A support office of eight full-time researchers, from post-doctoral students to intercalated students, underpins the highly active research environment at BCCH. This team enabled me to have five original articles under review and podium presentations during the year at the Paediatric Orthopaedic Society of North America (POSNA) and Canadian Orthopaedic Association (COA) annual congresses.

Within weeks of arrival I had submitted a funding proposal with Dr Cooper. We were awarded the Innovations in Acute Care and Technology (iACT) grant, which has funded our investigation into weight-bearing magnetic resonance imaging (MRI) of patients with Perthes disease. Vancouver has the world's only research upright MRI system, and our study is the first to utilise it for young children. The child can be imaged while standing, so unique images of femoral heads in early-stage Perthes disease under load can be obtained and compared with standard supine images. Early results indicate a dynamic deformity under load in Perthes disease, which could clarify the surgical indications for this disease. We developed the protocol for optimum MRI sequences and patient positioning, with my son being integral to that process as a healthy volunteer (Figure 1).

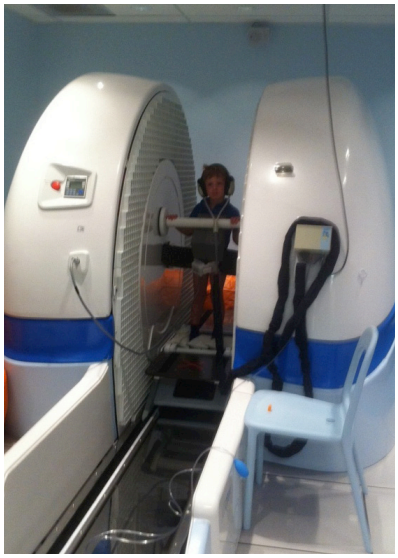

Figure 1. Benjamin Aarvold (aged 5 years) assisting with protocol planning in the unique research upright magnetic resonance imaging system scanner

A further example of the value of the research support is our novel article, written together with my co-fellows and Dr Alvarez, on acute ulnar lengthening for Madelung's deformity in hereditary multiple exostoses. Like so much literature in orthopaedics, this is a single-surgeon case series, but by far the largest published to date. The research support staff facilitated tracking of patients and data collection, allowing myself and my co-fellows to focus our time on data analyses and writing.

The International Hip Dysplasia Institute research database is run out of Vancouver under the directorship of Dr Mulpuri. I was able to analyse this multiple-national prospective

Cutner Travelling Fellowship

database with its 5 years of data. We presented findings on the prevalence of success of Pavlik harness for fixed dislocations at COA and POSNA. Furthermore, I was integrated into the IHDI committee and attended board meetings in Florida (USA) and Las Vegas (NV, USA).

SURGICAL TRAINING

Several training differences became apparent between the UK and North American systems. Canadian residents all undertake medicine as a postgraduate course, which is a 4-year degree. Subspecialty residency programmes are applied for, and started directly from, medical school. There are no foundation year/junior house officer/senior house officer grades. Orthopaedic training is a 5-year run-through programme starting from day 1 as a doctor. Eighteen months are spent in a basic surgical training scheme followed by 3 and a half years of orthopaedics. Thus, training is complete 5 years after graduation.

The training years are shorter, but the intensity is undoubtedly greater. Ward rounds are done before the start of the working day (usually at 6 am) ready for a start at 7am. Each session of residency posts is a clinic or theatre. There is no allocated time for research or study. On-calls overnight and at weekends do not have European Working Time Regulations time off *in lieu*. Less than full-time training is not permitted.

Figure 2. Past and present Fellows at British Columbia Children's Hospital with Professor Steve Tredwell and Professor Alain Dimeglio (seated)

VISITING PROFESSOR

BCCH was treated to a visit by Professor Alain Dimeglio from Montpelier (France) as the annual visiting professor. He gave lectures on his pioneering work on skeletal growth and management of club foot. He engaged in complex case discussions we had saved up for him. This annual day attracts many past fellows (Figure 2) and is focused on current fellows, who are entrusted to wine and dine the visiting professor throughout their trip.

CONCLUSIONS

To be immersed in a different healthcare culture can augment training. I have harnessed the benefits of the NHS and Canadian systems. I can use those experiences to be a better clinician, teacher, researcher and leader. This comes at considerable financial cost, however, and I am extremely grateful to the Royal College of Surgeons of Edinburgh for the support provided in the award of the Cutner Fellowship.

Travelling Fellowship Report

Cutner Travelling Fellowship

Mr James Ferguson

Travelled to Beit Cure International Hospital, Blantyre, Malawi

August 2014 to August 2015

I worked for 1 year at Beit Cure International Hospital (BCIH) undertaking a fellowship in paediatric orthopaedics. This work would not have been possible without the generous support of the Cutner Travelling Fellowship of The Royal College of Surgeons of Edinburgh. My family and I are immensely grateful for this opportunity.

HEALTHCARE IN MALAWI

Malawi is a landlocked country in south-eastern Africa with a population approaching 17 million. One-third of its land area is consumed by the third largest lake in Africa: Lake Malawi. Malawi has the dubious honour of being the poorest country in the world when measured by gross domestic product. Since independence in 1964 the country has remained relatively peaceful and its largely rural population relies on agriculture and subsistence farming. However, this is a nation where the overwhelming majority of people live hand-to-mouth with no societal 'safety nets'. About 40% of the government budget comes directly from international aid.

In the UK there is an average of 1 doctor for every 369 people, whereas in Malawi the figure is 1 doctor for every 50,000 people. There are nine orthopaedic surgeons in the whole of Malawi, seven of which work in Blantyre. There is no ambulance service and hospitals suffer additional challenges caused by intermittent supplies of power and water. The burden of trauma in Malawi is particularly high, with many individuals disabled through accident and injury.

Figure 1. A baby undergoing Ponseti casting for club foot being carried by his mother

Despite these challenges healthcare is free. Rural clinics and health centres across Malawi provide basic care, which is usually delivered by clinical officers. Individuals requiring hospital treatment are referred to regional district hospitals as required. At the top of this hierarchy are the four central hospitals, which provide more specialist care. The largest of these is the Queen Elizabeth Central Hospital (QECH) in Blantyre, which is situated just across the road from BCIH. The College of Medicine is also nearby; this was established in 1991 and is the only medical school in Malawi.

BCIH has been open for 13 years and its vision is to deliver high-quality orthopaedic care for children, such as for club foot (Figure 1). The hospital also offers orthopaedic services for fee-paying adults and is the only hospital in this region of Africa that undertakes joint replacement. Individuals come from far and wide to get treatment for arthritis or neglected trauma. This supplementary private work generates additional income for BCIH, which supports

Cutner Travelling Fellowship

the children's work. This strategy explains the motto emblazoned on its sign: "Adults pay a fee so the children can walk free". Although not yet achieved, this ethos marks BCIH as an organisation striving for a sustainable business model with a vision to eventually become self-sufficient and independent from reliance on charitable donations.

FELLOWSHIP

I had heard a lot of good things about BCIH having talked with several volunteers and getting to know one of the inspirational hospital founders. I was delighted to be accepted for the fellowship and, with my wife and two small children, embarked upon it.

We were received with warmth and I quickly felt like a valued member of the team. I was particularly struck by the community spirit evident from the hospital director through to the cleaning staff and drivers. BCIH benefits from being a relatively small institution with a clear ethos. It was a pleasure working in such an environment.

A TYPICAL WEEK

Five orthopaedic consultants work at BCIH: a Briton, American, Ugandan, Kenyan and Malawian. They work in two teams (Figure 2) to allow more efficient use of operating theatres. Alongside them there are two senior clinical officers who undertake some surgery and run many of the clinics. BCIH also forms part of the local training programme, with a local trainee rotating every 6 months. Operating lists run 4 days a week.

Figure 2. Carrying out soft-tissue release for club-foot deformity

A single anaesthetic consultant oversees the theatres but most of the anaesthetics are administered by anaesthetic clinical officers.

Children are seen in clinics, and any patient requiring surgery is booked in for their procedure. Patients are told (via their parents) to arrive on the Sunday afternoon to allow relevant preparations to be made for theatre. All patients admitted to hospital must bring a guardian who is responsible for their day-to-day care. Each guardian will stay with the patient throughout their inpatient stay, usually sleeping on a mattress next to the bed.

Each Monday, during the consultant ward round, all children are seen, and a final surgical plan is made based on a review of each case. Each child undergoes preoperative malaria screening and is checked for anaemia. Prevalence of malaria and malnourishment among children was surprisingly high. Sometimes, surgery was postponed to allow treatment of infection or malnourishment. In this situation, a high-protein diet is prescribed, usually comprising a daily egg to accompany the maize-based staple called 'nsima'.

Twice a week there is an 'open door' paediatric outpatient clinic that allows any child with an orthopaedic problem to be seen free of charge. Some children travel long distances to the clinic, some even from Mozambique. These clinics are supplemented by regular additional peripheral clinics across Malawi staffed by experienced orthopaedic clinical officers.

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The range of disease seen in Malawi is very different from the typical childhood problems encountered in the UK. Complications related to infections of bone and joint are common, as are angular deformities of the legs caused by rickets and other forms of malnourishment. Many children have cerebral palsy as a consequence of brain injury from malarial infection or birth trauma.

Other commonly encountered problems include children with club foot, spina bifida and congenital deformities. Sadly, BCIH also sees many children presenting with the late consequences of burns. This problem is encountered regularly because children are left in charge of open fires used by families to cook food. Children can fall into the fire, their clothing can catch alight or they may be scalded by hot liquids. These injuries rarely receive timely treatment, resulting in terrible scarring that contracts to leave limbs bent and useless, or joints dislocated, leading to severe disability.

In rural communities, the stigma associated with childhood orthopaedic problems is huge. Children with deformities are commonly ostracised and excluded from their communities. Attending school is difficult and these children often have to deal with these additional pressures beyond their physical disability. One of the greatest privileges of this year was being able to see the slow but wondrous transformation in these children. As their physical needs were addressed so their psychological healing began. To see these children gain confidence and come 'out of their shells' during their stay was fantastic.

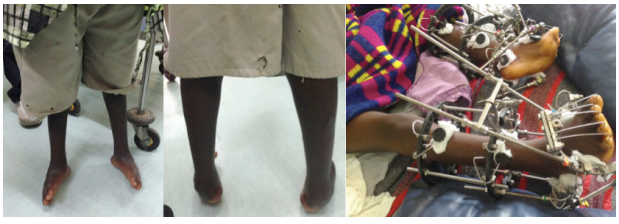

Figure 3. A child with neglected club-foot deformity. Correction was achieved with bilateral Ilizarov frames.

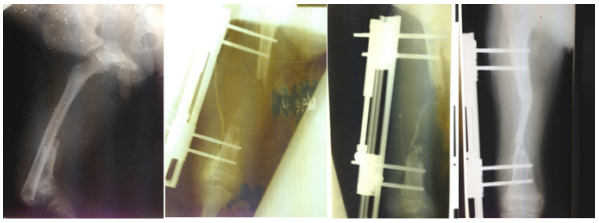

Figure 4. This child presented late after an open femoral fracture due to a fall from a mango tree. After debridement of dead bone and application of an external fixator, a large defect was present. The image on the right shows new bone formation in this defect as a result of the remaining periosteum.

I also spent some time visiting and working in the trauma department at QECH. A much larger proportion of operative cases were for non-unions, malunions and late complications, rather than for 'fresh' trauma. Dealing with the injured at QECH required ingenuity and re-acquaintance with methods now rarely used in the UK (eg skeletal traction for lower-limb fractures).

CASES

The majority of the operative cases I was involved with I had never encountered in the UK. I became familiar with the management of bowed-leg deformities in children. Straightening of legs was achieved acutely with corrective osteotomies of the tibia, or gradually with use of external fixation frames.

Club foot (Figure 3) and its long-term consequences were common. I learnt various surgical methods for this problem, from tendon transfer and soft-tissue release, through to osteotomies and frame correction (Figure 4).

I also became more familiar with plastic surgical methods (Figure 5). For burn contractures we made use of full-thickness skin grafting but also local flaps. Furthermore, I undertook several cases of syndactyly release as well as treating other congenital problems.

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I saw how chronic osteomyelitis is managed in a resource-poor environment and the surprising (but effective) use of honey as a potent antiseptic in surgical management of this disabling condition. Honey is poured into the bone void after excision of the infected sequestrum. Due to its high sugar content and some antibacterial effects it is a very effective treatment for infection.

Several children presented with limb deformities that had become severe because of a lack of treatment (Figure 6). This type of problem is seen rarely in the UK due to the availability of more aggressive early treatment. Often these deformities required the use of frames to achieve correction.

The trauma seen at QECH was very interesting. Late surgical treatment of injuries was common due to the volume of cases encountered. Use of image intensifier x-rays in theatre was scarce, so many fractures had to be opened to allow correct reduction.

TEACHING EXPERIENCE

I became involved in teaching quite quickly. I could see the importance of supporting training for healthcare workers, and wished to become familiar with their training programmes. An interesting distinction from the UK is that surgical specialties are not very popular in Malawi. Orthopaedics is among the most competitive disciplines in medicine in the developed world but uptake by trainees in Africa is poor. This difference can be explained (at least in part) by the lack of resources available in this specialty. Furthermore, much of the attention of charities and governments in the Western world has focused on communicable diseases, public health and child health, resulting in a comparative underinvestment in surgical disciplines. The need for future orthopaedic surgeons is urgent because of the overwhelming burden of morbidity and disability associated with neglected trauma.

In September, the annual Malawian Orthopaedic Association Conference took place on the shores of Lake Malawi. I was asked to give a

Figure 6. A girl with deformities of both legs from untreated Blount's disease, which was managed using Taylor spatial frames

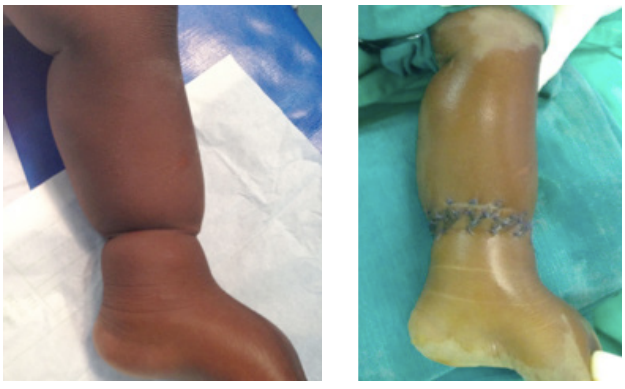

Figure 5. Plastic surgical methods. Amniotic band syndrome was treated with multiple z-plasties.

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presentation on complex limb reconstruction and acted as a facilitator for small group discussions.

In November, I was invited by the Association of Surgeons of Great Britain and Ireland to help run courses in Basic Surgical Skills and Training the Trainer in Victoria Falls, Zimbabwe. During this week, local surgical trainees were taught practical surgical skills, and consultant trainers were mentored in supervising trainees.

I was asked to teach fourth-year medical students and became involved in the surgical exams for this year group. I became a trainer on the BSc orthopaedic degree course set up for orthopaedic clinical officers. I was also asked to lecture physiotherapy undergraduates and supervised visiting medical students who had chosen Malawi as their elective base.

LIFE IN MALAWI

There were serious floods during the rainy season that affected large areas of the country. Buildings collapsed, leaving many injured or homeless. Furthermore, the maize fields that the population depend upon were damaged, raising concerns about food availability later in the year. These problems were compounded by the huge impact of deforestation, resulting in more severe flash floods and greater misery for people.

The country is beautiful and after being invited to go mountain-biking in the countryside around Blantyre I became hooked. The day starts early in Malawi, and we would leave at dawn. The surrounding area is very hilly and, although exhausting, the scenery was breathtaking. We encountered a large variety of wildlife on our travels, visiting the tea estates south of the city as well as hiking out onto the Zomba plateau.

Toward the end of our time in Malawi we were lucky to stay a few days up at Lake Malawi. As the sun sets, the lights on the dugout canoes of the fishermen can be seen from the shore, explaining why the Lake is often known as "the lake of stars". We were very lucky to get to know some wonderful people who supported us through the highs and lows of living in Africa.

CONCLUSION

Working in Malawi has given me a very different perspective on global healthcare. It has been humbling working at BCIH and seeing the dedication of the staff, even when facing the many challenges of delivering healthcare in a resource-poor setting. I have seen and learnt many new skills and gained in confidence in managing some challenging conditions.

The importance of institutions like BCIH has been underlined to me because of its commitment to engage with the training of local surgeons and clinical officers. I believe that supporting local trainees and doctors is essential if healthcare is to improve. This year was invaluable and I gained a lot of experience managing complex and interesting cases. Many of the skills I have learnt will help me in my UK practice.

Travelling Fellowship Report

Sir James Fraser Travelling Fellowship

Beatrix Elsberger

Clinical Lecturer in Surgical Oncology, Ninewells Hospital, Dundee

Visit to MD Anderson Cancer Center, Houston, Texas, USA

July 2015

MD Anderson Cancer Center (MDACC) is situated within the 'hospital district' of Houston (TX, USA). It has ranked as number one in cancer care in North America for more than 7 years running. It is the largest centre in the world devoted exclusively to the prevention, treatment and eradication of cancer.

MDACC occupies >14 million square feet and provides the latest technology and facilities to support outpatient and inpatient care, research and education. With my interest and background in surgical oncology (specifically breast cancer (BC)) I thought this would be an ideal institution to observe and study all aspects of cancer care. The BC unit of MDACC is a separate building with a team comprising 24 breast surgeons, an almost equivalent number of plastic surgeons, ≈40 medical oncologists and ≈10 radiation oncologists to deal with >4500 newly diagnosed patients with BC each year. To put this figure into perspective, our local BC unit has 3 breast surgeons managing 480 new patients with BC per year.

Each team member has published extensively on BC. I found myself walking alongside and discussing BC cases with the likes of Professor Kelly Hunt and Henry Kruerer, who have led the way in running clinical practice-changing BC trials. Irrespective of professional standing, everybody was very approachable and spent extra time in answering questions regarding management.

Only 25% of BC patients are from in and around Houston. Most patients treated at MDACC are from other American states (25%) or overseas (50%). When a private health insurance company gives permission to full entitlement the patient can choose where they wish to receive cancer treatment. In general, patients presenting to the MDACC breast clinics have been given their cancer diagnosis elsewhere. It is very much a selected BC cohort. Over 60% of BC patients I met during my visit were aged <40 years with rare and aggressive BC subtypes. This scenario provided an ideal environment to conduct translational research and run clinical trials for which multicentre international collaboration is usually needed. Each clinician is dedicated to recruit patients into trials.

Sir James Fraser Travelling Fellowship

The infrastructure and capacity of information technology (IT) is impressive. All patients have an assigned internet account, "My MDAnderson", accessible by all staff and by the patient to check appointments, investigations, and even clinical letters and communications. Patients seem to be very proactive, filling out personal details and uploading documents online. IT security is understandably very high because health history (including diagnostic and personal images) is available with one click. From a clinician's viewpoint this is an excellent setup for treatment planning.

MDACC has a high prevalence of requests (>40%) for bilateral mastectomy and immediate implant reconstruction from patients. These consultations were always challenging. It became clear that such requests from young women are based on immense fear. To rationalise and contest against this fear for life requires exceptional communication skills balancing the wishes and retaining the trust of the patient while quoting the literature and best clinical practice.

The breast service is led mainly by consultants. Each breast surgical consultant has their own mini-team of advanced nurse practitioner (ANP) and physician assistant (PA). The PA functions as a secretary, breast care nurse, junior doctor and assistant in theatre. They are the first port of call for the consultant's new patients and is in constant email contact with them. All pre-theatre investigations and work-up are completed by the first appointment. Therefore, based on the presentation summary given by the PA, decisions on surgical treatment can be made with the patient immediately. The well-known and established weekly multidisciplinary team meeting in the UK, in which each individual cancer case is discussed, is not feasible at MDACC due to the number of staff and different schedules. Each breast specialty (surgery, radiology, pathology, radiation oncology, medical oncology) conducts its own weekly case discussion. Only four breast surgical fellows join the team each year. Due to the reputation of MDACC with regard to training and teaching delivery, these posts are very much sought after.

Over 85% of all breast surgical procedures (including oncoplastic procedures) are handled as day cases. Admissions for overnight stays are required mainly for uncontrolled pain relief or unforeseen blood loss. After the immediate postoperative phase, patients tend to recover in nearby long-term rentable accommodation. This strategy is less expensive than additional overnight stays in hospital. Most patients have travelled long distances, so they are accompanied by relatives, who care and support them through their cancer treatment.

Surgical training in the USA is known for its long hours. A theatre day starts early: the first patient is on the operating table, intubated, and ready for the skin incision by 7am. Hence, a breast surgical fellow must be at theatre reception by 6 am at the latest for signing in, changing and starting all pre-theatre checks of listed patients. Most first cases are finished by 9am.

MD Anderson's main facility has >30 theatres. The five breast surgical theatres, on their own floor, run parallel Monday to Friday 7am to 10pm. Three breast surgeons operate each day and work alongside plastic surgeons. For example,

Sir James Fraser Travelling Fellowship

for a planned nipple-sparing mastectomy with immediate implant reconstruction, the breast surgeon starts the case with their PA and, as soon as the mastectomy is completed, the case is handed over to the plastic surgeon. The breast surgeon immediately moves into the theatre next door, where the next patient is anaesthetised, ready and waiting.

It has been demonstrated that a 'positive' attitude of patients is important for recovery from cancer treatment. Hence, MDACC's follow-up programme is called "cancer survivorship" and comes with its own nurse specialists, physicians and psychologists. Other precautionary strategies used by clinicians in MDACC are chemoprevention in the adjuvant setting and after the diagnosis of B3 lesions (atypical ductal hyperplasia is a high-risk lesion) and for risk reduction in women with a strong family history of BC. Implementation into UK practice for high-risk patients has been sporadic despite its recommendation as being part of guidelines from the National Institute of Health and Clinical Excellence. It is unclear if this phenomenon is due to low uptake from patients, lack of evidence on the timeline or pathology of the cancer itself because most BC-1 patients are oestrogen receptor-negative.

The most interesting aspect of my visit was to observe implementation of research innovations into clinical practice. MDACC is a world leader in conducting clinical trials, including development of new surgical methods (eg radiolabelled ^{125}I seed-guided excision of impalpable BC). Standard UK practice for these types of lesion is wire-guided excision, which can be challenging because the wire can be displaced. Radiolabelled ^{125}I seeds can also be placed in abnormal-looking axillary lymph nodes at time of the diagnosis of primary BC before administration of neoadjuvant chemotherapy to ascertain the effect of systemic therapy. This approach has given rise to a debate: is clearance of axillary nodes necessary for patients who have a full response to radiography?

During my time at MDACC it became clear that its motto "making cancer history" is not an empty promise. It addresses patients and cancer as a whole, and is an excellent example of how cancer centres should be run. I must give a very special thank you to Professor Alastair Thompson and Dr Stacey Moulder, who made my stay at MDACC immensely enjoyable and worthwhile.

Travelling Fellowship Report

John Steyn Travelling Fellowship in Urology

Dr Pankaj, Senior Resident, Department of Urology, Chandigarh
Department of Urology, University Hospitals of North Midlands, Stoke on Trent

I would like to thank Mr Anurag Golash for giving me this opportunity to work at the University Hospitals of North Midlands (UHNM). UHNM is located at Grindley Hill Court and is well connected to the rest of Stoke on Trent. UHNM is a huge building with a helipad for air ambulances.

The urology department has 8 consultants, 4 registrars and a few interns. Mr Golash is the head of the department. Most of my time was spent under 2 consultants: Mr Golash and Mr Fernando. The former is renowned for introducing day-care laparoscopic nephrectomy into UK practice. Mr Fernando has good expertise in flexible ureteroscopic procedures. I also had the opportunity to observe preoperative preparation, taking of written informed consent, the World Health Organization checklist, and patient care during the perioperative period.

I observed: 6 laparoscopic radical nephrectomies; several flexible ureteroscopic procedures; laser intracorporeal lithotripsy; transurethral resection of the prostate gland; open cystolithotomy; laparoscopic adrenalectomy for phaeochromocytoma; double-J stenting; circumcision. I also sat in clinics with Mr Golash, where I observed his interaction with patients, learning how to explain problems, break bad news to patients, and how the referral system works. Attending clinics also gave me very good insight into how compassionate and attentive we must be to patient needs. The diagnosis may be identical, but every patient is unique in their response to the disease or treatment, and we must tailor our approach to suit their needs.

I also attended departmental multidisciplinary team (MDT) meetings, which was a totally new experience. In MDT meetings we discussed difficult cases and how to better manage them by involving radiologists and other specialists. I attended ward rounds and learned about the care of bed-bound patients. I had the opportunity to interact with other departments (eg breast care).

I would like to extend many thanks to Mr Golash and his team for giving me this wonderful opportunity to work in their department. I hope I shall be able to provide care of the highest standard to my patients based on my experience at UHNM.

Ophthalmology Grant Reports

Ophthalmology Grant Report

Development of photoreceptor transplantation in the totally degenerate retina

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

Major Project Grant
1 October 2013 to 30 September 2014

LAY SUMMARY

Retinitis pigmentosa (RP) is one of the primary causes of inherited retinal blindness. In RP, an initial loss of rod photoreceptors promotes secondary degeneration of cone photoreceptors. Photoreceptor transplantation could be a future treatment for retinal diseases such as RP that result in end-stage retinal degeneration (ie no photoreceptor cells remain) by repopulating the degenerate outer nuclear layer (ONL) with photoreceptors.

We have developed a photoreceptor transplantation method in laboratory mice and have demonstrated that transplanted photoreceptors can survive and integrate with the residual host retina. We now wish to optimise the method so as to enable transplanted cells to reconstitute a uniform and integrated ONL that restores visual function to the mice. Results thus far have been very promising and demonstrate that this application would be highly relevant to development of treatments for RP and other diseases leading to photoreceptor loss.

GRANT REPORT

(A) CLINICAL AND SCIENTIFIC SIGNIFICANCE OF ADVANCES MADE

Stem-cell therapy remains a long way from being a treatment for patients with inherited retinal degeneration. Nevertheless, this year saw the release of the preliminary results of the first clinical application of stem cells in a retinal pigment epithelium (RPE) cell-replacement trial led by the company Ocata Therapeutics: stem cells could be introduced safely into the retina without causing significant inflammatory reactions or teratoma formation.

RPE cell-replacement therapy has applications for retinal diseases caused by RPE degeneration, such as age-related macular degeneration and Stargardt disease. However, many retinal diseases such as RP are caused by the malfunction or death of photoreceptors, for which photoreceptor progenitor cell-replacement therapy would be required.

The vast majority of experiments in photoreceptor transplantation to date have not been done in 'true' models of human retinal degeneration in that the mouse hosts used in widely cited literature have a nearly full complement of photoreceptors at transplantation. A key breakthrough would be to repeat these

Development of photoreceptor transplantation in the totally degenerate retina

experiments in the totally degenerate retina because this approach would equate to the clinical scenario. Hence, our current work in photoreceptor transplantation has concentrated on developing a method for reconstructing an entirely new ONL.

In this study, we have optimised the transplantation method in a mouse model of end-stage retinal degeneration. We have shown that large numbers of surviving photoreceptors that reform an ONL can be generated. Many of the factors affecting the survival and integration of photoreceptors remain unresolved, but this study has permitted development of an efficient method of photoreceptor transplantation in murine models. As a result of our expertise in this domain, Ocata Therapeutics has entered into a very promising collaborative research project with our group at the University of Oxford with the aim of collecting sufficient preclinical data to warrant clinical trials.

(B) PROBLEMS ENCOUNTERED AND STEPS TAKEN TO OVERCOME THEM

We have sought to maximise formation of new connections from transplanted cells to the degenerate host retina by various means, such as removal of subretinal debris before transplantation by pre-treatment with tissue plasminogen activator (tPA). We have shown that donor photoreceptor cells can reconnect to the residual host retina, but pre-treatment with tPA does not obviously improve this interaction. A better approach may be to prevent potential formation of glial scars resulting from the inflammatory response mediated by Müller cells.

(C) COLLABORATIONS ESTABLISHED

Oxford Stem Cell Institute

The Oxford Stem Cell Institute is providing collaborative assistance to our research team for investigation of the regenerative capacities of stem cells for the treatment of retinal disease – a promising area of research of which the potential therapeutic benefits cannot be overemphasised. The support given to our research team is being channelled into the development of methods to guide differentiation of human stem cells into immature photoreceptors of the correct stage of development to render them suitable for transplantation into host retinas.

Ocata Therapeutics

Ocata Therapeutics (formerly named Advanced Cell Technology) is a clinical-stage biotechnology company focused on the development of new therapies in regenerative medicine. The company's principal research efforts are invested in regenerative ophthalmology, and specifically in the development of stem-cell therapies for the treatment of eye diseases and disorders. Ocata Therapeutics is conducting the world's first clinical trial of a RPE cell-replacement therapy for retinal diseases caused by RPE degeneration.

Development of photoreceptor transplantation in the totally degenerate retina

Ocata Therapeutics is also seeking to develop photoreceptor progenitor cell-replacement therapy for retinal degenerations caused by the malfunction or death of photoreceptors. In this regard, Ocata Therapeutics is conducting a very promising collaborative research project with our research team at the University of Oxford, and building upon our experience and expertise in photoreceptor progenitor transplantation in murine models. A great deal of our recent work in photoreceptor progenitor transplantation arises from this research project funded by The Royal College of Surgeons of Edinburgh.

Results thus far have been very encouraging, and were presented in a poster entitled "Transplantation of *ex vivo* genetically modified photoreceptor precursors: *in vivo* improvement in visually guided behaviour following transplantation of AAV transduced cells" at the 2014 conference of the Association for Research in Vision and Ophthalmology (ARVO). It is our goal to collect sufficient preclinical data to warrant clinical trials.

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

HIGHER DEGREES AND PRIZES

Three DPhil students – Mandeep Singh, Alona Cramer and Sher Aslam – received prizes for their work on this research project funded by The Royal College of Surgeons of Edinburgh under guidance of the postdoctoral research scientist who was supported by the award.

MANDEEP SINGH

- Ruskell Medal, Worshipful Company of Spectacle Makers
- Founder's Cup, Oxford Ophthalmological Congress

ALONA CRAMER

- Members-in-training (MIT) Outstanding Poster Award in the Nanotechnology and Regenerative Medicine Cross-Sectional Group for her presentation on photoreceptor transplantation at the 2014 ARVO conference
- Young Researcher in Focus, European Vision Research

SHER ASLAM

- Finalist in ARVO MIT Outstanding Poster competition

PUBLICATIONS

The publications arising from this research project, and acknowledging the financial support of The Royal College of Surgeons of Edinburgh, are listed below.

- Singh MS, Charbel Issa P, et al. Reversal of end-stage retinal degeneration and restoration of visual function by photoreceptor transplantation. *Proc Natl Acad Sci USA* 2013; 110: 1101–1106

Development of photoreceptor transplantation in the totally degenerate retina

- Cramer AO, MacLaren RE. Translating induced pluripotent stem cells from bench to bedside: application to retinal diseases. *Curr Gene Ther* 2013; 13: 139–151
- Cramer AO, Singh MS, McClements ME, et al. Transplantation of *ex vivo* genetically modified photoreceptor precursors: *in vivo* improvement in visually guided behaviour following transplantation of aav transduced cells. *ARVO* 2014; Abstract 1446
- Singh MS, Aslam SA, Duncan I, et al Cell fusion following photoreceptor transplantation into the non-degenerate retina. *ARVO* 2014; Abstract 3989
- Aslan SA, Barnard AR, Sekaran S, et al. Cone transplantation. *ARVO* 2014; Abstract 3981

Additional manuscripts are in preparation. Other publications, arising from previous research projects supported by The Royal College of Surgeons of Edinburgh, were also published during this period. These publications, which all acknowledged the support of The Royal College of Surgeons of Edinburgh, are listed below.

- Aslam SA, Yusuf IH, MacLaren RE. Unsutured phakic implantation of a black intraocular lens in the sulcus to treat leukocoria. *J Cataract Refract Surg* 2014; 40: 1565–1567
- MacLaren RE, Groppe M, Barnard AR, et al. Retinal gene therapy in patients with choroideremia: initial findings from a phase 1/2 clinical trial. *Lancet* 2014; 383: 1129–1137
- Aslam SA, Davies WI, Singh MS, et al. Cone photoreceptor neuroprotection conferred by CNTF in a novel *in vivo* model of battlefield retinal laser injury. *Invest Ophthalmol Vis Sci* 2013; 54: 5456–5465
- Perganta G, Barnard AR, Katti C, et al. Non-image-forming light driven functions are preserved in a mouse model of autosomal dominant optic atrophy. *PLoS One* 2013; 8: e56350
- McClements ME, MacLaren RE. Gene therapy for retinal disease. *Transl Res* 2013; 161: 1–21

FURTHER FUNDING

An application to the Medical Research Council for funding of £946,000 to support a research project into the mechanism of retinal-cell fusion has been submitted recently on the strength of the preliminary data obtained from this research project supported by Tthe Royal College of Surgeons of Edinburgh.

(E) ACKNOWLEDGEMENTS

We thank The Royal College of Surgeons of Edinburgh and Royal Blind for their generous sponsorship of this and previous Major Project Ophthalmology Grants, by which our retinal research programme has been assisted greatly.

Ophthalmology Grant Report

In vitro evaluation of immunological properties of crosslinked recombinant human collagen hydrogels used in corneal regeneration for pre-clinical applications

Professor John V Forrester
School of Medicine and Dentistry, Institute of Medical Sciences, Section of Immunology, Inflammation and Infection (Ocular Immunology), Division of Applied Medicine, University of Aberdeen

Major Project Grant

1 June 2014 to 31 May 2015

LAY SUMMARY

There is a gap between the millions suffering from corneal blindness and the availability of donor corneas for replacement of opaque corneas. Novel strategies have been proposed to address this problem. One approach is to use hydrogels that simulate the composition and qualities of natural human corneas and which are transparent (a critical feature for light to enter the eye). To succeed, hydrogels would need to: (i) integrate well with the surrounding cornea; (ii) remain clear long-term (like a normal cornea would). The body rejects foreign objects, so artificial corneas lose the clarity needed for sight.

We are studying immune cells known as 'dendritic cells' (DCs) that help the body decide if the foreign object poses a threat. If DCs 'see' something as 'dangerous', the body expels it. If DCs ignore the foreign object, the body allows it to remain and function. We believe that culturing DCs on hydrogels and examining their responses will give us valuable clues as to whether the body is likely to reject the hydrogel in an animal model. Our study will predict promising hydrogels as candidates for artificial corneas. The long-term goal would be to advance affordable corneal alternatives in patients worldwide.

GRANT REPORT

(A) CLINICAL AND SCIENTIFIC SIGNIFICANCE OF ADVANCES MADE

Background

Corneal blindness affects millions worldwide and the only therapeutic option is corneal transplantation. Unfortunately, the availability of viable human corneas is a limiting factor. As regenerative medicine gains prominence, novel approaches to regenerate corneas from biointeractive implants have been proposed. Specifically, recombinant human collagen (RHC) III hydrogels designed to mimic collagen I and III present in natural corneas and crosslinked to resist biodegradation maintained clarity post-implantation in lamellar[1] or penetrating[2] keratoplasties in mini-pigs or rabbits. Importantly, a 4-year clinical study of lamellar keratoplasty of N-(3-dimethylaminopropyl)-N′-ethylcarbodiimide (EDC)-N-hydroxysuccinimide (NHS) hydrogels demonstrated enhanced visual acuity and minimal rejection with regenerated and stably integrated neo-corneas.[3] The specific mechanisms driving immune acceptance or rejection remain to be elucidated. DCs are antigen-presenting cells that link innate and adaptive immunity.[4] DC responses to corneal transplants may determine the balance between host immunity versus tolerisation and hence influence the fate of corneal grafts. DCs are recruited from the bone marrow during

In vitro evaluation of immunological properties of crosslinked recombinant human collagen hydrogels used in corneal regeneration for pre-clinical applications

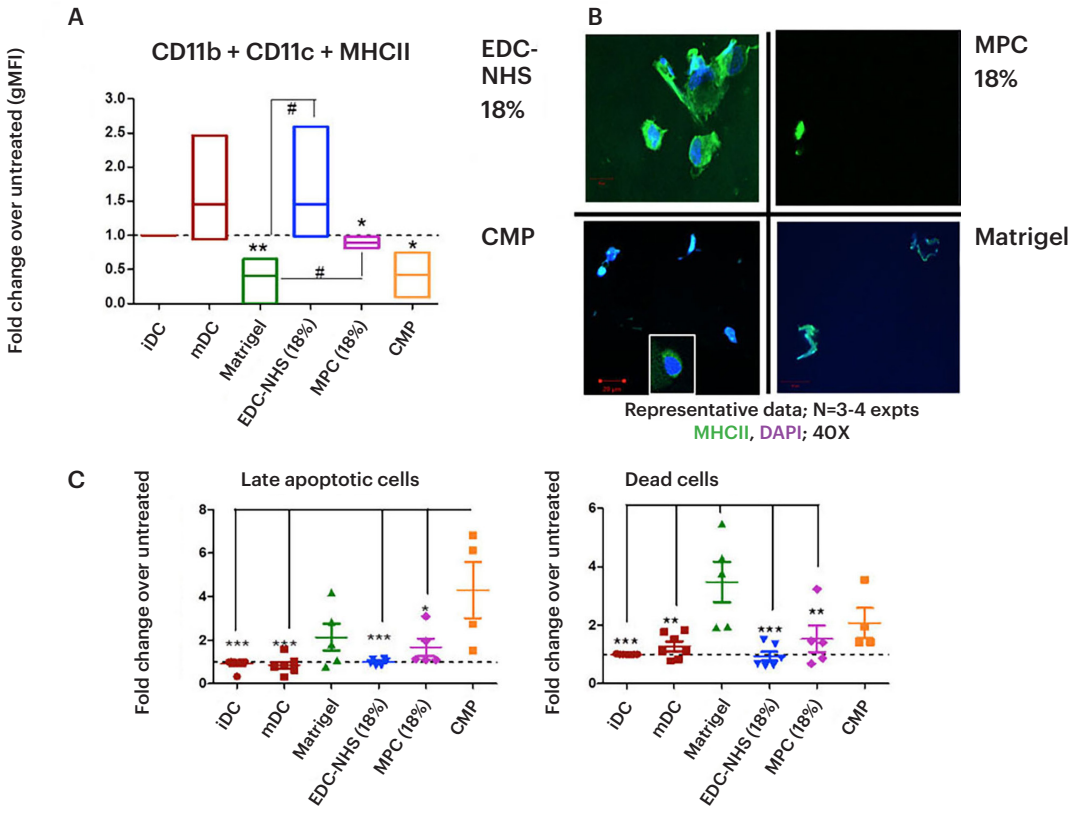

Figure 1. Differential responses of dendritic cells (DCs) to hydrogels with different crosslinking chemistries after 24-h culture. Decreased levels of MHCII on CD11b+ CD11c+ DCs cultured on Matrigel, MPC (18% RHCIII) or CMP hydrogels as compared with untreated iDCs (mean ± SEM; N=4–7,'*'p≤0.05, ''p≤0.001 unpaired t-test). In contrast, elevated expression of MHCII was detected on CD11b+ CD11c+ DCs cultured on EDC-NHS (18% RHCIII) ('#'p≤0.05, unpaired *t*-test) (A). Elevated surface expression of MHCII for DCs treated with EDC-NHS indicative of maturation in contrast to counterparts on CMP or Matrigel (representative data, N=3) (B). Increased presence of late apoptotic or dead cells on CMP hydrogels or Matrigel, respectively, as compared with other treatments (C) (mean ± SEM; N=4–7,'*'p≤0.01, '**'p≤0.001, '***'p≤0.0001 one-way analysis of variance with Tukey post test).**

rejection of corneal grafts, and are thought to have different roles from the resident tolerising DCs in the conjunctiva/cornea of the eye.[5] The hypothesis underlying this study is that examining DC responses to differentially crosslinked hydrogels *in vitro* may help predict their immune acceptance *in vivo* after implantation in murine corneas. Hydrogels that activate DCs minimally may be beneficial in corneal regenerative applications.

Results summary

Murine bone marrow-derived DCs were cultured on differentially crosslinked RHCIII hydrogels for 24 h to examine the effects of chemistry, charge or hydrophobicity on DC maturation. DC activation was assessed in terms of maturation markers (cluster of differentiation (CD)83, CD86 (B-7 family of costimulatory molecules)), major histocompatibility class (MHC)II) and apoptosis

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levels by flow cytometry and cellular morphology by confocal microscopy. Interestingly, hydrogels associated with minimal levels of DC maturation were associated with highest apoptosis of DCs and elevated presence of cellular debris (Figure 1). Specifically, DCs cultured on Matrigel (mimics the basement membrane) or collagen mimetic peptide (CMP) hydrogels appeared least activated in terms of lower expression of CD83, CD86 or MHCII versus negative control of untreated immature DC, shown here for MHCII (Figures 1A, B) and also increased presence of late apoptotic or dead cells, respectively, as compared with all other treatments (Figure 1C). Phagocytosis of apoptotic DCs on these hydrogels may have had a tolerogenic effect on DC. In contrast, DCs cultured on EDC-NHS (18% RHCIII) hydrogels displayed elevated levels of DC activation and decreased levels of DC apoptosis (Figures 1A, B). Finally, DCs cultured on hydrophilic 2-methacryloyloxyethylphosphorylcholine (MPC) hydrogels (18% RHCIII) (mimic of phospholipids in plasma membranes) were activated modestly (Figure 1A) and were present sparsely within hydrogels (Figure 1B).

Murine corneas underwent penetrating keratoplasties with EDC-NHS or hydrophobic CMC-NHS hydrogels and were assessed 22 days post-transplantation (pt)[6]. Hydrogels themselves remained clear and supported re-epithelialisation of corneas after implantation, but they were encapsulated by fibrous capsules that blocked light transmission and vision[6], demonstrating that the host immune/inflammatory responses to artificial corneas are complex. Both types of hydrogels were rejected, but more recent work ongoing from this study suggests that the underlying mechanisms involved are dissimilar. CD11c+ DCs and CD11b+ monocyte/macrophages could be detected in re-epithelialised, keratinised corneas as well as in the retro-corneal membranes 22 days pt in EDC-NHS transplanted eyes (Figure 2A), but CD11c+ DCs could not be visualised in N-cyclohexyl-N′-(2-morpholinoethyl) carbodiimide metho-*p*-toluenesulphonate (CMC)-NHS counterparts (Figure 2B) because of varying kinetics of the cellular responses or contributions from other immune cells. DCs could be visualised within CMP transplanted hydrogels as early as 6 h pt and are shown here at 24 h pt (Figure 2C), evidence that they may have crucial roles in defining the immune response.

Interestingly, extracellular matrix (ECM)-driven remodelling occurred with both types of hydrogel transplants, as seen for α-smooth muscle actin produced by activated myofibroblasts as part of the wound-healing process (Figure 3A, B), though to differential extents for tenascin C (Figure 3C, D), a marker for epithelial-to-mesenchymal transition produced during the early stages of wound healing. Interestingly, CMC-NHS hydrogels which did not support recruitment of DCs at day 22 were associated with higher positive staining for tenascin C (Figure 3D) versus EDC-NHS transplanted eyes (Figure 3C), implying a possible inverse correlation between DCs and lowered expression of tenascin C. Cytokeratin 12 could be detected in re-epithelialised membranes of both types of transplanted hydrogels, shown here for CMC-NHS transplanted corneas (Figure 3E). Overall, these observations underscore the regenerative potential of artificial corneas.

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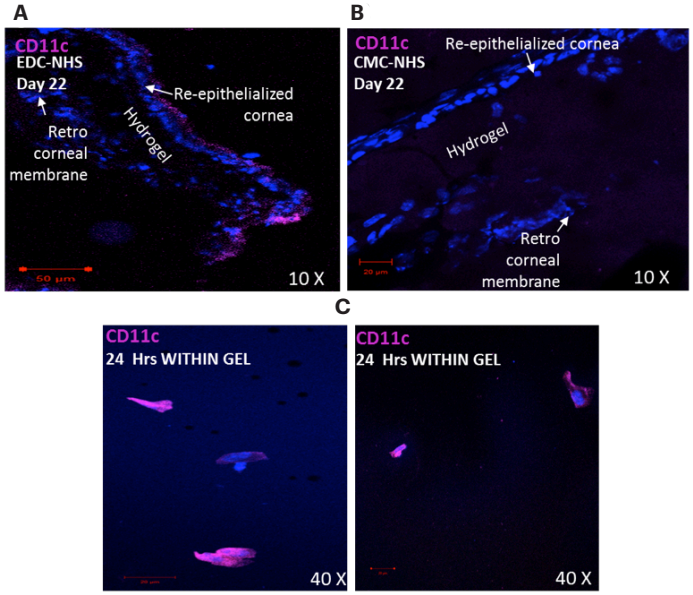
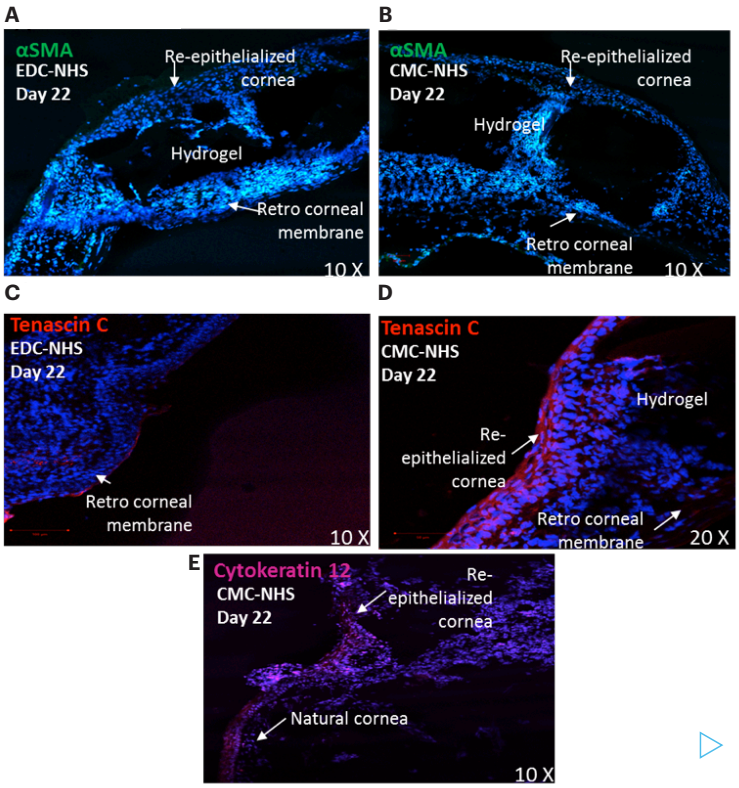


Figure 2. Dendritic cells (DCs) have a role in host responses to RHCIII hydrogel transplants in murine corneas. DCs are recruited to murine corneas transplanted with EDC-NHS hydrogels at 22 days pt and are present in re-epithelialised corneas and at gel edges (A), but are not detectable in CMC-NHS transplanted corneas (representative data, N=5) (B). DCs are also present within transplanted CMP hydrogels at 24 h (C). DAPI nuclear staining is shown in blue. This figure has been adapted in part from Shankar SP, Forrester J, Kuffova L. Dendritic cells and the extracellular matrix: a challenge for maintaining tolerance/homeostasis. *World J Immunol* 2015; 5: 113–130.

Figure 3. EDC-NHS or CMC-NHS RHCIII hydrogels transplanted into murine corneas are well integrated at 22 days pt. Staining corresponding to αSMA (merged green) (A, B) and tenascin C (red) (C, D) in the newly generated corneal epithelium, fibrous capsule posterior to the hydrogel, or cellular ingrowths into the hydrogel demonstrate active remodelling of the bioscaffold. Corneal cytokeratin 12 (magenta) (E) indicates formation of a terminally differentiated epithelium (representative data, N=5). DAPI nuclear staining is shown in blue. This figure has been adapted in part from Shankar SP, Forrester J, Kuffova L. Dendritic cells and the extracellular matrix: a challenge for maintaining tolerance/homeostasis. *World J Immunol* 2015; 5: 113–130.



In vitro evaluation of immunological properties of crosslinked recombinant human collagen hydrogels used in corneal regeneration for pre-clinical applications

Conclusions

Overall, the microenvironment provided by the hydrogel matrix orchestrates the migration, infiltration, maturation and survival of DCs and, therefore, affects downstream adaptive immune responses, which are crucial for promoting graft functionality in host corneas.

Future directions

The next steps will be to elucidate the cellular mechanisms underlying differential DC responses and to establish which components of hydrogels contribute to DC activation. Hydrogels associated with lower activation of DCs from *in vitro* studies such as CMP- or MPC-crosslinked gels will be taken forward in *in vivo* studies of murine corneal transplants.

Scientific importance

The major advances made so far are:

- Demonstration of the important role of DCs in mediation of the host response to hydrogel transplants of murine corneas.
- Establishment of an *in vitro* screening system that enables rapid and high-throughput prediction of successful hydrogel candidates for artificial corneas based on their interactions with DCs. This approach is beneficial compared with animal models, which are relatively expensive, time-consuming and lower throughput.

Clinical importance

This study will generate criteria for the optimal design of ECM-based bioscaffolds such as artificial corneas that can support wound healing optimally, integrate with host tissue and remain functional, for pre-clinical and clinical applications. The ultimate objective would be to fabricate hydrogels that are likely to be well-accepted by the host and retain their clarity and long-term functionality for use in patients.

(B) PROBLEMS ENCOUNTERED AND STEPS TAKEN TO OVERCOME THEM

Scientific

- **Optimisation of the culture system:** A successful DC–hydrogel culture system has been established using a 96-well plate setup with hydrogel discs trephined to fit exactly within wells and optimisation of DC seeding density to achieve consistent results.
- **Keeping hydrogels sterile for DC culture:** A consistent protocol involving treatment of hydrogels with antibiotics and fungicides to preserve sterility after trephining and subsequent maintenance in cell-culture media to ensure absence of contaminants is being followed stringently. Levels of endotoxins or other contaminants are being assessed.
- **Alternative to collagenase treatment of hydrogels:** Instead of characterising DCs that have infiltrated hydrogels by collagenase treatment of gels for several hours to ensure complete degradation, an alternate method of fixing the hydrogels first and then staining fluorescently and visualising DCs within the gel by confocal microscopy has been used. This change has overcome the problem of lengthy collagenase treatments which may damage cells, and has also allowed visualisation of three-dimensional interactions of unmanipulated cells with the collagen matrix.

Logistical

Preparation of hydrogels was technically challenging initially in terms of equipment and personnel expertise, which resulted in temporary delays to supplies. These problems have been resolved and a steady supply of hydrogels established.

In vitro evaluation of immunological properties of crosslinked recombinant human collagen hydrogels used in corneal regeneration for pre-clinical applications

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5. Forrester JV, Xu H, Kuffová L, et al. Dendritic cell physiology and function in the eye. *Immunol Rev* 2010. 234: 282–304

6. Ahn JI, Kuffova L, Merrett K, et al. Crosslinked collagen hydrogels as corneal implants: effects of sterically bulky vs. non-bulky carbodiimides as crosslinkers. *Acta Biomater* 2013; 9: 7796–7805

(C) COLLABORATIONS ESTABLISHED

May Griffith, Professor of Regenerative Medicine and Director, Integrative Regenerative Medicine Centre, Linköping University, Department of Clinical and Experimental Medicine, Division of Cell Biology, Linköping, Sweden

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

Publications

Shankar SP, Forrester J, Kuffova L. Dendritic cells and the extracellular matrix: a challenge for maintaining tolerance/homeostasis. *World J Immunol* 2015; 5: 113–130

Yu T, Rajendran V, Griffith M, et al. High-risk corneal allografts: a therapeutic challenge. *World J Transplant* 2016; 6: 10–27

Further funding

The Royal College of Surgeons of Edinburgh, Major Project Ophthalmology Grant, 2015–2016 (£49,960).

(E) ACKNOWLEDGEMENTS

We acknowledge The Royal College of Surgeons of Edinburgh and Royal Blind for funding received during 2014–2015. We thank the confocal microscopy and flow cytometry core facilities at the University of Aberdeen for technical assistance. We are grateful to staff at the Medical Research Facility for their expertise with animal handling.

Ophthalmology Grant Report

Development of transplantation of cone photoreceptors

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

Major Project Grant
1 October 2014 to 30 September 2015

LAY SUMMARY

Transplantation of photoreceptor precursors (ie immature photoreceptor cells that have not yet developed into mature rod or cone photoreceptors) could be a future treatment for patients with blindness or low vision due to retinal degenerative diseases.

In the first year of this 2-year research project supported by The Royal College of Surgeons of Edinburgh, we developed a method of transplantation of photoreceptor precursors in laboratory mice. We demonstrated that the transplanted photoreceptor precursors could survive and integrate with the residual host retina, leading to eventual regeneration of the entire outer nuclear layer. This work was recognised widely to represent a major advance in development of stem-cell therapies for retinal disease, and was published in the broad science journal *Proceedings of the National Academy of Sciences USA*, with an accompanying editorial in the journal on the significance of this advance.

The second year of this 2-year research project was to go one step further and replicate the mouse studies but this time using human cells. To do this, we established a collaboration with Ocata Therapeutics, a stem-cell company based near Boston (MA, USA) which implemented the first retinal stem-cell clinical trial in 2008 (Schwartz et al., *Lancet* 2012; Schwartz et al., *Lancet* 2015).

GRANT REPORT

(A) CLINICAL AND SCIENTIFIC SIGNIFICANCE OF ADVANCES MADE

We remain extremely grateful to the Royal College of Surgeons of Edinburgh for the grant support that has enabled us to pursue a highly successful translational research programme into developing treatments for incurable blindness. In the last year we have expanded our retinal gene-therapy trials to the USA and Canada, using the same vector developed in part through one of the previous grants from the College. Together with the Wellcome Trust, the University of Oxford has formed NightstaRx Limited, a retinal gene-therapy that has attracted £40 million in venture capital funding which will be used to run an international multicentre gene-therapy trial and gain regulatory approval. We have, therefore, demonstrated success not only in developing laboratory treatments, but also in attracting the follow-on funding and expertise needed to push these exciting scientific developments into real treatments for patients.

Development of transplantation of cone photoreceptors

Background to current project

Cone photoreceptors are lost early in age-related macular degeneration (AMD), which is the leading cause of visual impairment in people aged >50 years and which affects ≤500,000 people in the UK. AMD causes progressive loss of central vision, which eventually prevents afflicted individuals from being able to drive, to read a book/computer screen, to watch television, or even to recognise faces. The goal of this 2-year research project has been to develop a photoreceptor cell-replacement therapy for treatment of retinal degenerations, and particularly the loss of cone photoreceptors responsible for central vision.

Stem-cell therapy for photoreceptor regeneration

Ocata Therapeutics (formerly named Advanced Cell Technology) is a clinical-stage biotechnology company developing stem-cell therapies for treatment of diseases and disorders of the eye. Types of retinal stem cells under investigation are induced pluripotent stem cells (iPSCs) and embryonic stem cells (ESCs).

Through the collaboration with Ocata Therapeutics, we have been able to work with human cells for the first time. This is always a critical point on the translational pathway because, though the mouse studies show excellent proof of concept, it is ultimately human cells that we would wish to transplant. We, therefore, assessed the therapeutic potential of human photoreceptor progenitors derived from human ESCs and iPSCs using a protocol suitable for future regulatory approvals towards clinical trials. The human ESCs and IPSCs were labelled with a green fluorescent protein (GFP) using a viral vector expressing the gene isolated originally from the bioluminescent jellyfish *Aequorea victoria*. Then, ESCs and IPSCs identified by their GFP expression were transplanted into blind mice with a naturally occurring inherited retinal degeneration (rd1) similar in phenotype to end-stage retinal degenerations in humans.

After transplantation of ESCs and IPSCs (fluorescent green) into the degenerate retinas of *rd1* mice, these cells were observed to

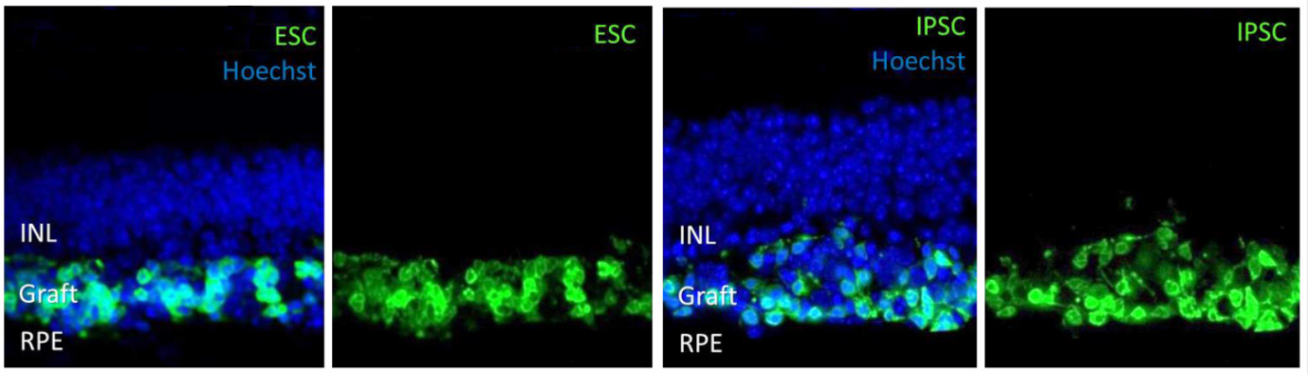

Figure 1. Results showing transplantation of ESCs and IPSCs (fluorescent green) into the degenerate retinas of *rd1* mice.

Development of transplantation of cone photoreceptors

differentiate into photoreceptors and form a cell layer connected with host retinal neurons. Figure 1 shows that the transplanted fluorescent green ESCs and IPSCs form a new outer nuclear layer ('Graft') that has fully integrated with the inner nuclear layer ('INL', stained blue with Hoechst dye) of the degenerate retinas of host *rd1* mice.

Figure 1 shows surviving human ESCs and iPSCs regenerating an outer nuclear layer. Human cells are green and images are shown with and without a nuclear label (Hoescht stain) to identify retinal layers. The pattern of differentiation and size of graft is similar in ESC- and iPSC-derived cells. Basic visual function was restored in treated animals, as evidenced by behavioural light-avoidance tests. These data validate the potential of human pluripotent stem cells for photoreceptor replacement therapies and reveal similar efficacy using ESCs or iPSCs as source materials. The manuscript summarising these experiments has been submitted for publication.

(B) PROBLEMS ENCOUNTERED AND STEPS TAKEN TO OVERCOME THEM

To our great interest, we observed that the mechanism by which transplanted photoreceptor precursors integrated with the degenerate retinal layers of the host mice involved transient cell fusion. 'Cell fusion' refers to a process whereby a hybrid cell is formed containing two nuclei that carry the genetic material of each cell alongside a common cytoplasm.

To provide additional experimental data that cell fusion was occurring, evidence needed to be obtained that 'true' hybrid cells were being formed that contained the nuclei from each of the two parent cells. To this end, photoreceptor precursor cells were extracted from the eyes of newly born female mice (ie having XX sex chromosomes), and then we injected them into the degenerate retinas of male *rd1* mice (ie having XY sex chromosomes). Several of the integrated photoreceptors were shown to have a Y chromosome, which would have been impossible for photoreceptor precursors of female origin unless cell fusion had occurred.

We are continuing our investigative work in this interesting phenomenon. Cell fusion has significant potential to be harnessed as a therapeutic reprogramming mechanism in stem cell-based approaches in the regeneration and repair of retinas.

(C) COLLABORATIONS ESTABLISHED

Ocata Therapeutics

Ocata Therapeutics is a clinical-stage biotechnology company focused on the development of new therapies in regenerative medicine. The company's principal research efforts are invested in regenerative ophthalmology, and specifically in the development of stem-cell therapies for the treatment of diseases and disorders of the eye.

Development of transplantation of cone photoreceptors

Ocata Therapeutics is conducting the first clinical trial of a retinal pigment epithelium (RPE) cell-replacement therapy for retinal diseases caused by RPE degeneration.

Ocata Therapeutics is also seeking to develop a photoreceptor progenitor cell-replacement therapy for retinal degenerations caused by the malfunction or death of photoreceptors. In this regard, Ocata Therapeutics is conducting a very promising collaborative research project with our team at the University of Oxford, and building upon our experience and expertise in photoreceptor progenitor transplantation in murine models. A great deal of our recent work in photoreceptor progenitor transplantation arises from this research project funded by The Royal College of Surgeons of Edinburgh.

Results thus far have been very encouraging, and a manuscript on transplantation of photoreceptors derived from human pluripotent stem cells, using data collected over the course of this 2-year research project, has been submitted by Ocata Therapeutics and Professor MacLaren to the journal *Stem Cells*.

Johns Hopkins University

Dr Mandeep Singh, the ophthalmologist working under Professor Robert MacLaren who developed this technique during his DPhil, has been appointed to the Faculty as an Assistant Professor in the Retina Division of the Wilmer Eye Institute at the Johns Hopkins Hospital (Baltimore, MD, USA).

Dr Mandeep is continuing his valuable collaboration with Professor MacLaren, and an additional manuscript on the subject of photoreceptor cell fusion, using the data collected during the second year of this 2-year research project, has been submitted by Professor Singh and Professor MacLaren to the journal *Nature*.

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

Higher degrees and prizes

- Mandeep Singh and Alona Barnea-Cramer, the DPhil students who assisted Dr Alun Barnard (the postdoctoral research scientist supported by this award) over the course of this 2-year research project, have graduated with DPhil degrees from the University of Oxford.
- Dr Barnea-Cramer has just taken up her first post-doctoral position working on stem cells with the Nobel Laureate Professor Paul Greengard at Rockefeller University (New York, NY, USA). Her appointment to Rockefeller University was earned in part from her work on the retinal stem-cell project, highlighting the high calibre of the research undertaken with support of the Royal College of Surgeons funding.

Prizes received by Professor Singh over the course of this 2-year research project:

- Ruskell Medal, Worshipful Company of Spectacle Makers
- Founder's Cup, Oxford Ophthalmological Congress

Prizes received by Dr Barnea-Cramer over the course of this 2-year research project:

- MIT Outstanding Poster Award in the Nanotechnology and Regenerative Medicine Cross-Sectional Group for her presentation on her work on photoreceptor transplantation at the 2014 ARVO conference
- Young Researcher in Focus, European Vision Research (2014)

Development of transplantation of cone photoreceptors

Publications

The key publication submitted during the first year of this 2-year research project, and acknowledging the financial support of The Royal College of Surgeons of Edinburgh, is shown below.

- Singh MS, Charbel IP, Butler R, et al. Reversal of end-stage retinal degeneration and restoration of visual function by photoreceptor transplantation. *Proc Natl Acad Sci USA* 2013; 110: 1101–1106

Additional manuscripts, based on the data collected during the second year of this 2-year research project, are being drafted. The financial support of The Royal College of Surgeons of Edinburgh is acknowledged in the following manuscripts which have been submitted recently for publication.

- Barnea-Cramer AO, Wang W, et al. Functional assessment of transplanted photoreceptors derived from human pluripotent stem cells. *Stem Cells*
- Singh MS, Balmer J, Aslam SA, et al. Cell fusion in photoreceptor transplantation. *Nature*

Other publications, arising from previous research projects supported by The Royal College of Surgeons of Edinburgh, have been published during the second year of this 2-year research project. These publications, which all acknowledged the support of The Royal College of Surgeons of Edinburgh, are listed below.

- Barnard AR, Groppe M, MacLaren RE. Gene therapy for choroideremia using an adeno-associated viral (AAV) vector. *Cold Spring Harb Perspect Med* 2014; 5: a017293
- Edwards TL, Groppe M, Jolly JK, et al. Correlation of retinal structure and function in choroideremia carriers. *Ophthalmology* 2015; 122: 1274–1276
- Lipinski DM, Barnard AR, Singh MS, et al. CNTF gene therapy confers lifelong neuroprotection in a mouse model of human retinitis pigmentosa. *Mol Ther* 2015; 23: 1308–1319
- Charbel IP, Barnard AR, Herrmann P, et al. Rescue of the Stargardt phenotype in Abca4 knockout mice through inhibition of vitamin A dimerization. *Proc Natl Acad Sci U.S.A.* 2015; 112: 8415–8420

(E) ACKNOWLEDGEMENTS

We thank The Royal College of Surgeons of Edinburgh and Royal Blind for their generous sponsorship of this and previous Major Project Ophthalmology Grants, which have underpinned the successful translational research programme in Oxford. We are pleased to report that our early laboratory work has now been developed into international clinical trials and it remains our commitment to research new treatments for currently incurable blindness. To date we have made considerable progress in that regard.

Ophthalmology Grant Report

A randomised controlled trial to reduce retinal displacement and symptoms of distortion after repair of retinal detachment

David Charteris

Moorfields Eye Hospital

Major Project Grant

June 2014 – ongoing

LAY SUMMARY

Distorted vision after surgery for retinal detachment is common, but there is no good evidence as to how it is best prevented. We plan to compare two commonly used approaches to head positioning after surgery to ascertain if either has a particular advantage over the other by reducing symptoms of distortion and improving vision after surgery. Trial participants will also be followed up for 6 months after surgery to determine if early symptoms of distortion, and associated structural changes in the healing retina, persist over time and are of functional importance to the patient.

Study participants will have surgery as normal but will have 1–2 more follow-up appointments and additional non-invasive investigations. Study results will, in the future, help retinal surgeons advise patients on optimal postoperative care.

GRANT REPORT

(A) CLINICAL AND SCIENTIFIC SIGNIFICANCE OF ADVANCES MADE

Since the grant award in 2014 our study team has refined the trial protocol and raised an additional £161,000 for infrastructural funding to enable the study to proceed. A research fellow (Edward Casswell) took up his post in February 2016 and the clinical trial commenced shortly afterwards. A research nurse and research coordinator has been allocated to the study.

(B) PROBLEMS ENCOUNTERED AND STEPS TAKEN TO OVERCOME THEM

Infrastructural costs have been identified and £16,100 of additional funding (Moorfields Eye Hospital Special Trustees) generated to cover them. The study team applied to the Integrated Research Application System for ethics approval in December 2015.

(C) COLLABORATIONS ESTABLISHED

The study has established a collaboration between the vitreoretinal surgery units at the two trial centres: Moorfields Eye Hospital (London) and the Tennent Institute of Ophthalmology (Gartnavel Hospital, Glasgow). The lead researcher in Glasgow (Dr David Yorston) has developed a metamorphopsia measurement system (the D-Chart) which will be used by both centres in the study.

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

The research fellow will register for a higher degree based on work in this study.

Ophthalmology Grant Report

Modulation of pathogen receptor signalling in human bacterial keratitis

Mr Parwez Hossain

Clinical Experimental Sciences, Faculty of Medicine, University of Southampton

Major Project Grant

1 March 2014 to 28 February 2015

LAY SUMMARY

The bacterium *Pseudomonas aeruginosa* (PA) is the principal cause of bacterial keratitis worldwide. Overstimulation of the innate immune system by this organism is a major factor for sight loss.

With support from the Royal College of Surgeons of Edinburgh we have shown how PA interacts with the cornea using an *ex vivo* model of human infection. We determined that key elements of the bacterial components (flagellin and type three secretion system (TTSS)) interact with corneal cells during initial stages of the infection. These components stimulate the innate immune system via inflammasome activation.

Most importantly, we can show that flagellin activates the inflammasome pathway via the TRAM–TRIF pathway. This key pathway results in the release of inflammatory mediators called cytokines (eg interleukin (IL)-18 and IL-1β). We found that IL-18 expression is wholly dependent on flagellin sensing by extracellular PA, and that IL-18 and IL-1β are expressed and processed via the key inflammasome activators NLRC4/caspase-4.

Discovering this mechanism provides us with a basis for developing targeted therapies to interrupt inflammatory processes during PA infection by targeting interactions in flagellin signalling. Now, with additional support from the College, we have started to investigate this therapeutic option.

Our findings have been submitted to the microbiology journal *Virulence*.

GRANT REPORT

(A) CLINICAL AND SCIENTIFIC SIGNIFICANCE OF ADVANCES MADE

Infection or inflammation of the cornea is a major cause of visual impairment. Interactions between infectious agents and host tissue trigger an immune response which, in most cases, leads to excessive inflammation. This scenario results in severe visual impairment that is often refractory to current treatments.

PA is the leading Gram-negative bacterium implicated in corneal infections associated with wearing of contact lenses and ocular trauma. Expression of cell wall lipopolysaccharide (LPS) O-antigen, a TTSS and flagellin are key PA antigenic factors. LPS is a classical inducer of host inflammatory processes and the TTSS is an injection system composed of PopB, PopD and PcrV molecules (essential proteins for the exotoxin delivery). Possession of the TTSS has been suggested to be indispensable for the PA strain PAO1 to promote tissue damage and keratitis in animal models. Flagellin expression contributes to virulence by facilitating the motility and adhesion of PA.

Toll-like receptors (TLRs) are anchored, cell-surface pattern recognition receptors (PRRs) that sense pathogen-associated molecular patterns (PAMPs) and trigger the signalling cascades for expression of pro-inflammatory molecules. Corneal epithelial cells from mice and rabbits express TLR2, TLR4 and TLR5. TLR2 senses bacterial lipoproteins and lipopeptides whereas TLR4 is activated by LPS. Although expressed

Modulation of pathogen receptor signalling in human bacterial keratitis

constitutively in cells, we have demonstrated previously (from College funding in 2008) that TLR4 expression increases in a dose-dependent manner in response to high levels of LPS from PA in an *ex vivo* model of human corneal infection using primary corneal fibroblast cells (hCFs) (*Investigative Ophthalmology and Visual Science*, 2011). During the inflammatory response, it is believed that resident stromal keratocytes transform into hCFs, which have a key role in remodelling and healing of the cornea. LPS has also been shown to activate intracellular murine caspase-11 and human caspase-4 directly. TLR5 senses the extracellular flagellin (FliC) component, which can be delivered within the cytosol.

Nod-like receptors (NLRs) are cytosolic PRRs that sense intracellular PAMPS and promote assembly of a multiple-protein scaffold called the 'inflammasome'. The assembled inflammasome recruits and activates certain caspases for maturation of IL-1β and IL-18 pro-forms, inducing a cell-death programme called 'pyroptosis'. IL-1β is a pleiotropic cytokine that can mediate auto-inflammatory diseases and induce a wide range of inflammatory responses. IL-18 was originally described as an interferon-δ inducer and, though its role in different diseases has been demonstrated, its activity is strictly dependent on binding of its inhibitor, the IL-18 binding protein (IL-18BP). The canonical NLRP3 and non-canonical NLRC4 are the two inflammasomes reported to assemble in the corneal stroma in mouse models. NLRP3 transcription results from signalling cascade(s) triggered by TLR stimulation, whereas NLRC4 activation occurs by a critical phosphorylation of Ser533 residue and favours caspase-11 recruitment in mouse models. The caspase-11 homologue in humans is caspase-4.

Our current knowledge regarding flagellin and TTSS from PA as determinants for inflammasome stimulation is based on animal models: little is known about inflammasome activation in human eye tissue. In this project, we show how PA activates the innate immune response in an *ex vivo* model of corneal infection and show the critical role of stimulation of the TRAM–TRIF pathway by flagellin sensing for expression of IL-18 and IL-1β in humans. Furthermore, we show that NLRC4-activation as well as production of IL-1β and IL-18 is dependent on PA01 FliC protein. These results suggest NLRC4-alternative pathways for production of IL-18 and IL-1β in PA-based keratitis.

(B) PROBLEMS ENCOUNTERED AND STEPS TAKEN TO OVERCOME THEM

The main problem was identification of the key virulence factors in PA involved in inflammasome activation. This hurdle was overcome by using knockout strains of PA lacking key virulence factors (eg flagellin (FliC protein)) and using complementation methods to 'reconstitute' the organism.

(C) COLLABORATIONS ESTABLISHED

- Professor Alice Prince (Columbia University, New York, NY, USA)
- Professor George O'Toole (Geisel School of Medicine, Dartmouth College, Hanover, NH, USA)

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

- Taube MA, del Mar Cendra M, et al. Pattern recognition receptors in microbial keratitis. *Eye (Lond)* 2015; 11:1399–1415
- del Mar Cendra M, Christodoulides M, et al. Flagellin-mediated TRAM–TRIF pathway stimulation is critical for IL-18 and IL-1β production in human *Pseudomonas aeruginosa* keratitis. *Virulence* (manuscript in preparation)

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King James IV Professorship Lectures

King James IV Professorship Lecture

Emerging infections in health, disease and oral healthcare

Professor Crispian Scully
Emeritus Professor of Special-needs Dentistry, University College London

This lecture encompasses ongoing research interests in infections relevant to orofacial health, disease and healthcare (particularly viruses).

1. INTRODUCTION

Significant changes in lifestyle, social and healthcare have led to the emergence of several infections, particularly the human immunodeficiency virus (HIV) and the resultant acquired immune deficiency syndrome (AIDS). Immunosuppressive therapy is used increasingly in a range of conditions, especially in transplantation, to prevent T-cell rejection. Immunodeficiencies and immunosuppression lead to immunoincompetence and a liability to infections and malignant disease.

Infections are mainly due to mycobacteria, fungi and viruses. They are often recurrent and may spread rapidly, be clinically silent, be atypical, and transmissible in saliva. In particular they affect the skin, mucosae and respiratory tract. Neoplasms include melanoma, basal cell carcinoma, Kaposi sarcoma, lymphomas/lymphoproliferative disorders, as well as carcinomas of the skin, lip, genitalia, and perineum. Many new primary immune defects are now recognized, and we (our research team) have described their orofacial features and have been involved in classifying lesions in HIV/AIDS.

2. VIRUSES AND DISEASE

2.1. Herpesviruses

Herpesviruses are DNA viruses contracted typically in early life, transmitted in saliva, characterised by latency, and can be reactivated during immunosuppression. Herpesviruses often cause orofacial disorders.

The herpes simplex virus (HSV)-1 can cause oral or oropharyngeal infection, and is most prevalent in resource-poor groups. HSV-2 can cause severe oropharyngeal infection, usually via orogenital or oro–anal sexual contact. HSV-2 is more common in the sexually active, particularly among female sex workers and men who have sex with men.

The varicella-zoster virus (VZV) causes chickenpox but, perhaps more importantly, also shingles (zoster) mainly in older or immunocompromised patients. Zoster is a fairly common feature in HIV-infected patients treated with anti-retroviral agents who develop immune reconstitution inflammatory syndrome.

Patients with immune defects are liable to severe and/or protracted and/or disseminated herpesvirus infections: HSV, VZV, Epstein–Barr virus (EBV), cytomegalovirus (CMV) or Kaposi sarcoma herpesvirus (KSHV; eg HHV-8). We have described their involvement and management in various lesions.

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2.2. Human papillomavirus (HPV)

Papillomas, common warts (verruca vulgaris), genital warts (condyloma acuminatum) and focal epithelial hyperplasia (Heck disease) are caused by different HPVs. A higher prevalence of infection is seen in immunocompromised patients or those with sexually shared infections (SSIs). In the mouth, papillomas are uncommon, typically affecting fauces, soft palate or tongue. Warts are rare, usually transmitted from skin lesions, found predominantly on the lips, or from genital/anal lesions, and found mainly on the tongue or palate. We have demonstrated their viral aetiology.

2.3. Viruses and potentially malignant disorders and cancer

Oral cancer appears to be increasing in incidence, and we were the first to show an increase in Britain and the epidemiology in Scotland (where it is more frequent) and confirmed increases worldwide. Oral cancer is the result of DNA mutations arising spontaneously and from the action of various mutagens, mainly tobacco, betel and alcohol. We revealed an association with viruses that other scholars have confirmed since. We first demonstrated RNA complementary to HSV in lip cancer and a new human papillomavirus (HPV) in oral carcinoma. Our and other, more recent studies have shown changes in oncogenes and tumour suppressors, especially those involving 3p, in oral carcinoma.

Potentially malignant (premalignant or precancerous) disorders which precede some neoplasms include erythroplasia (erythroplakia) – the most likely lesion to progress to severe dysplasia or carcinoma – leukoplakia, lichen planus and submucous fibrosis. Our studies on HPV found the virus in some premalignant disorders and we have examined potential pathogenic mechanisms.

Based on this work, we hypothesised that oral cancer might be a SSI, a hypothesis vindicated by other researchers, mainly in oropharyngeal cancer. Research on HPV as well as oral and oropharyngeal cancer placed us in an exciting position because HPV vaccines became available, and the question as to whether these influence the rising incidence (especially in the young) has yet to be answered. Nevertheless, most cases of oral cancer continue to be related to tobacco and alcohol, and ongoing studies support this hypothesis.

2.4. BLOODBORNE VIRUSES (BBV)

2.4.1 HIV/AIDS

Infection with RNA retroviruses (eg HIV) results in infection that eventually damages T-cells, ultimately causing AIDS. CD4+ T-cells are crucial to host defences against fungi, viruses, mycobacteria and parasites, and HIV disease predisposes to infection with them. In the early days of the HIV epidemic, we showed the range of oral lesions (mainly infections and malignancies) as well as the reluctance of dental heathcare staff to care for HIV-infected people (despite the low risk of transmissibility and lack of complications related to oral healthcare procedures). We also showed the role of various herpesviruses and other infective agents.

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2.4.2 Hepatitis viruses

Hepatitis B, D and C viruses are among BBV transmitted in blood, blood products and other body fluids, particularly if there is needle- or syringe-sharing or skin breaches. Outbreaks have also occurred in healthcare: dental and other outpatient settings, haemodialysis units, long-term care facilities, and hospitals. These outbreaks have arisen primarily from unsafe injection practices, needle re-use, fingerstick devices, syringes and other lapses in infection control. Infection with the hepatitis-B virus has long been of greatest importance. We conducted a series of studies to highlight this fact, the prevalence of other forms of hepatitis, and the need for routine immunisation, glove use and infection control which slowly (but surely) helped campaigns designed to introduce infection control and immunisation. Standard precautions against infection transmission are universal so, in the absence of a vaccine, the hepatitis-C virus (HCV) has become the major issue, and we have demonstrated orofacial sequelae from HCV infection (eg lichen planus, Sjögren syndrome).

3. FUNGI

3.1 Candidosis

Approximately 50% of the population are '*Candida* carriers'. This pathogen grows opportunistically as yeasts or hyphae, mainly in people with immune defects. Thus, candidosis is a 'disease of the diseased'. The importance of candidosis increased greatly as the HIV pandemic extended. In immunocompromised people, *Candida* typically colonises mucocutaneous surfaces (commonly oropharyngeal) and can result in invasive candidosis. We have shown its role in HIV/AIDS and other immune defects, such as Down syndrome, and have examined aspects of its aetiopathogenesis in rats, as well as therapeutics.

3.2 Paracoccidioidomycosis

Caused by *Paracoccidioides brasiliensis*, paracoccidioidomycosis was seen mainly in South America (especially Brazil) before global travel. However, we have shown the importance of orofacial lesions, aetiopathogenetic mechanisms and the increased association with HIV and travel.

3.3 Histoplasmosis

Histoplasmosis is found worldwide. The causal agent, *Histoplasma capsulatum*, is present in the faeces of birds and bats. Disseminated and potentially fatal histoplasmosis is seen typically in immunocompromised patients (especially in those with HIV/AIDS). Orofacial lesions may be indicative of histoplasmosis.

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4. BACTERIA

4.1 Dental infections

We have studied bacterial pathogenesis in gingivitis and periodontitis in animal models, in patients with HIV/AIDS and other immune disorders, as well as the pathogenesis and therapy of dental abscesses.

4.2 Syphilis

Syphilis, a SSI caused by *Treponema paflidum*, is transmitted by direct contact with lesions via vaginal, anal, or oral sexual contact. Oral lesions have been reported.

4.3 Leprosy

Endemic in Asia, Africa, and Latin America, leprosy is also seen occasionally in southern Europe. The outcome of infection with *Mycobacterium leprae* is dependent upon immune reactions which, if intact, result in a localised form (tuberculoid leprosy) but, if deficient, cause generalised (lepromatous) leprosy and may cause orofacial lesions.

5. PARASITES

5.1 Leishmaniasis

Since the appearance of the HIV/AIDS pandemic, more parasitic infestations are being recognised. Leishmaniasis, common in the tropics and around the Mediterranean, can cause skin or orofacial lesions, especially in HIV/AIDS.

5.2 Myiasis

Myiasis occurs if fly maggots invade living tissue, or if they are harboured in the intestine or any part of the body and feed on the host's organs. Human myiasis is most common in tropical climates and, as we have reported, may affect the mouth, mainly where hygiene is defective (as in some resource-poor situations).

5.3 Larva migrans

Larvae burrow through tissue and may produce a type of creeping eruption. If they mature, they migrate and may then become visible (even in the mouth).

6. INFECTION CONTROL

Our research on dental healthcare infections (particularly hepatitis viruses, herpesviruses, and HIV) was, I believe, pivotal to the national and international introduction and uptake of immunisation, infection control and promotion of oral healthcare of people with HIV/AIDS. It led to personal involvement in producing guidance from the British Dental Association, General Dental Council and the National Institute for Health and Care Excellence.

King James IV Professorship Lecture

Promoting bone union and preventing fracture non-union

Professor Hamish Simpson
University of Edinburgh

King James the Fourth was a highly enlightened monarch. He was a patron of the arts and established the first printing press in Scotland in 1507. He was also a patron of science and medicine. In 1506 he granted the Royal Charter to the College of Surgeons of Edinburgh, which he had founded the previous year. Of particular note was his habit to practise as a dental surgeon.

It is particularly fitting to give this lecture on a European stage because James the Fourth had a European outlook. He spoke seven languages (including Latin, French, Flemish, German and Spanish) and had a Danish mother and an English wife.

He signed treaties of perpetual peace with France and England. The treaty with England led to the union of the crowns in 1603 when his grandson, the Scottish king, also became the English king.

During the Italian wars, James the Fourth was aligned with Venice and France. This brought him into conflict with England, who was aligned with the papal army and Spain. As a result of these allegiances, in 1513 England and Scotland fought each other in the battle of Flodden. James the Fourth led from the front and was killed tragically on the battlefield. He was the last British Monarch to die on the battlefield.

Fracture non-union is an ancient disorder: it has been recognised in archeological remains from more than 12,000 years ago. However, it is a constantly changing condition. This lecture will cover the changing aspects of this subject that are relevant to all of us. In particular, it will cover the incidence and classification of non-union, discuss new concepts concerning the causes of non-union, and end with how treatment may evolve in the future.

Previous estimates of the incidence of non-union have not been able to use large population data for all ages and for all sites of the skeleton. All patients in Scotland have a unique identifying number, so we were able to retrieve data on all non-unions. Over a 5-year period there were just under 5,000 non-unions. From these data the overall incidence of non-union was calculated and found to be 19 per 100,000 (Figure 1)[1], which is slightly higher than that for renal cancer and about one-third that of breast cancer.

The bones most at risk of progressing to non-union were the tibia, clavicle and humerus (Table 1)[2]. Non-union was rare in the axial skeleton. It was also notable that multiple non-unions were exceptionally rare in patients with multiple

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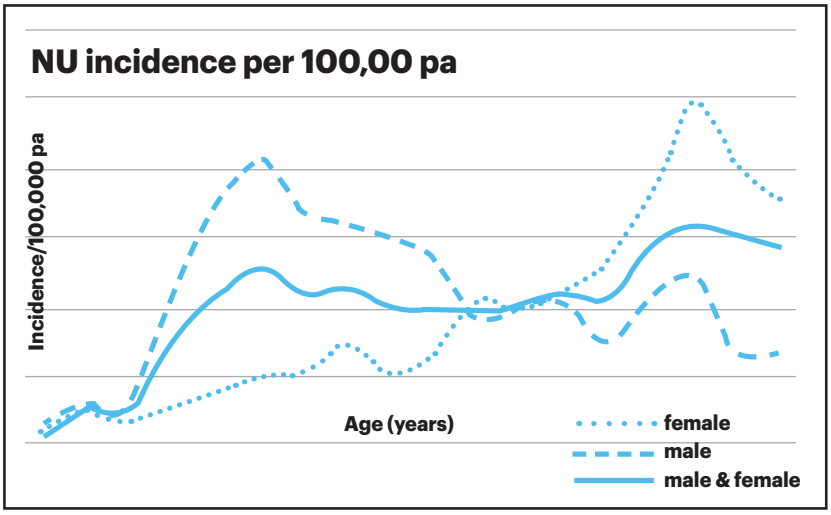

Figure 1. Incidence of fracture non-union in Scotland

fractures, which suggests that genetic influences are not a prime driver of non-union. The highest risk of developing a non-union was in the age range 30–44 years and not in the elderly.

Non-unions are commonly divided into 'hypertrophic' and 'atrophic' categories. A common dogma is that hypertrophic non-unions are vascular and active, and that atrophic non-unions are avascular and inert.

However, there is a problem in using radiography to infer the biology of non-unions, which we demonstrated by obtaining biopsy specimens from the fracture gap of patients with aseptic hypertrophic non-union, atrophic non-union, and from patients during normal fracture healing. Biopsy specimens showed that the vessel count in atrophic and hypertrophic non-unions was identical.[3] Further studies demonstrated that the cells retained their biological activity.[4, 5]

With hypertrophic non-unions, radiographs are a reasonable indicator of the biology because these non-unions are vascular and active. Atrophic non-unions have a similar appearance on radiography to the oligotrophic group but their biology is very different. Ilizarov recognised that the stiffness of the non-union site was an indicator of the connection between the bone ends: the stiff ones connected with vascular 'fibrous' tissue and the mobile ones with a false joint. Distinguishing between these two types is critical because the stiff ones are vascular and active, whereas the mobile ones are avascular and inactive. At surgery, fluid is found between the bone ends and a false capsule is present. The majority (probably 85%) of the atrophic/oligotrophic group are stiff.

The gap tissue from stiff atrophic non-unions has a range of cells and, under appropriate conditions, there are cells present that can differentiate into osteoblasts, chondrocytes or adipocytes. In particular, osteochondral progenitors are present within the gap tissue.[6] These cells, given the appropriate mechanical prescription, divide and differentiate along an osteoblast lineage to form bone.

Atrophic non-unions are known to have dead bone at the fracture site. However, even in patients with a simple closed fracture there is a small amount of bone death at the fracture ends. Therefore, we decided to examine the hypothesis that there was a critical length of dead bone at the fracture site that resulted in non-union. A controlled amount of the bone was

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	0–14 years	15–29 years	30–44 years	45–59 years	60–74 years	75+ years	Total	Adults
Clavicle	0.02	3.54	8.40	7.55	8.41	2.51	3.08	5.77
Humerus	0.15	3.58	5.20	3.09	3.49	2.21	2.44	3.08
Radius and ulna	0.08	4.13	3.36	1.30	0.92	0.44	0.78	1.87
Hand	0.02	0.33	0.28	0.18	0.16	0.05	0.23	0.25
Pelvis and femur	0.63	2.18	4.46	3.32	1.62	0.49	1.07	1.08
Tibia	0.20	4.80	7.16	6.17	4.48	2.44	2.28	5.17
Foot and ankle	0.26	0.67	1.00	1.06	0.90	0.99	0.84	0.91
Females	0.12	1.48	2.09	1.73	1.54	0.74	1.00	1.31
Males	0.09	1.38	1.95	1.53	1.72	0.90	1.00	1.54
Total	**0.10**	**1.39**	**1.99**	**1.62**	**1.60**	**0.77**	**1.00**	**1.43**

Table 1. Percentage risk of non-union per fracture according to age and anatomical distribution or sex

devascularised by stripping the periosteum and endosteum for gradually increasing amounts either side of the osteotomy. Despite making the bone dead for several centimetres either side of the osteotomy, all the bones united.

Therefore, in a second group of experiments, a small (but not critical) gap was created. If the periosteum/endosteum was left intact, all of these bones united. However with both a gap and a small amount of bone devascularisation, the bones suffered non-union, suggesting that a double insult was needed to produce non-union in these models.[7–9]

Reliable repair of fractures is of key importance for survival. For such a vital repair pathway, additional backup pathways are likely to ensure that healing occurs even in the presence of an inhibitor.

To investigate this concept further, we studied 100 consecutive non-unions and determined the causes for each patient under the headings of 'mechanical', 'infection' and 'bad biology', which could be a host factor or a combination of dead bone and a gap (Figure 2).[10]

Within the bad-biology category there were 7 cases considered to have dead bone and a gap as their sole cause and 5 cases considered only to have host factors.

However, 75% had more than one cause for non-union. The additional causes are not always obvious.[11] For instance, latent infection may present with a failure of fracture healing but no systemic toxicity in the same way that patients with loose joint replacements present with the symptoms of loosening rather than overt sepsis. It is, therefore, essential to collect multiple bacteriology samples and tissue for histopathology[12] routinely in non-union patients because in our cohort 6% of infected patients were not known to have any infection (systemic or local).

Infection inhibits fracture repair, so it is important to eradicate or suppress infection until the fracture has healed. However, certain antibiotics inhibit the repair process. Quinolones

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such as ciprofloxacin have been shown to inhibit the repair process in cell culture and animal models. Other antibiotics (eg rifampicin) and high local doses of gentamicin inhibit bone cells in culture. In contrast, the penicillins and vancomycin do not inhibit fracture repair.[13, 14]

Indirect repair of fractures uses acute-inflammation pathways. We, therefore, investigated the role of the immune system in fracture repair. Surprisingly, we found that in KO mice with no T-cells or B-cells there was accelerated fracture repair.[15] This finding was confirmed in an elegant study by Toben and colleagues in a different type of KO mouse.[16] More recently we have shown that the relative amounts of T-cell subsets (in particular the cluster of differentiation (CD)4 and CD8 cells) have a major effect on fracture repair.[17] CD4 cells are perturbed in patients infected with the human immunodeficiency virus (HIV) or those suffering from rheumatoid arthritis (RA). We, therefore, looked at the incidence of non-union in our RA patients, and found it to be significantly higher.[18] This finding may be due to their abnormal immune system. However, RA patients are often on numerous medications such as non-steroidal agents and these, rather than alteration of the immune system, may be responsible for the altered biological response to fracture. Whatever the cause, our study demonstrates a higher prevalence of non-union in RAA patients, which needs to be taken into account when treating their fractures because they may benefit from adjunctive therapy.

In contrast, direct repair of fractures does not rely on acute inflammation, but is led by

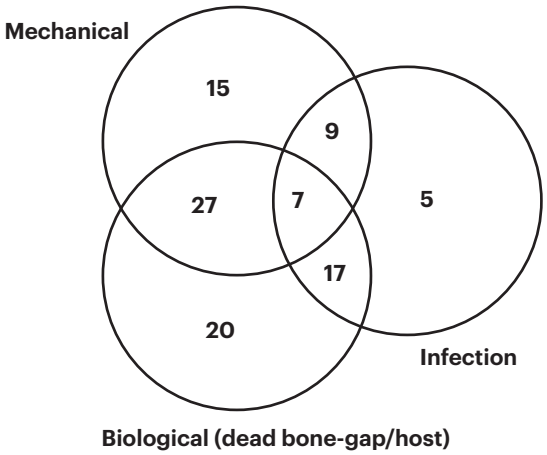

Figure 2. Distribution of causes of non-union

osteoclasts, which are the target cells for bisphosphonates. We, therefore, tested the hypothesis that bisphosphonates affect direct fracture healing adversely. A group of rats were randomised to receive ibandronate or saline.[19, 20] Bisphosphonates caused a profound inhibition of direct fracture repair (Figure 3). Hence, if an patient with osteoporosis sustains a fracture, if a direct repair process is being used, then it would be prudent to delay starting the drug. For patients already on bisphosphonates, we should choose a fixation method that allows the fracture to heal with secondary repair or we should stop bisphosphonates during the period of direct fracture healing.

Smoking has been shown to be detrimental to the healing of tibial fractures in several studies. For instance, a 2.3-fold greater prevalence of non-union has been reported in smokers compared with non-smokers.[21]

We have also shown an inhibitory effect of smoking in distraction osteogenesis (DO) patients in a multicentre study carried out in Edinburgh, Belfast, Bristol and Liverpool. The study was designed to ascertain if exogen accelerated healing in DO. No significant effect of ultrasound was observed in these DO patients, but smoking was found to reduce the prevalence of healing rate by 50%.[22]

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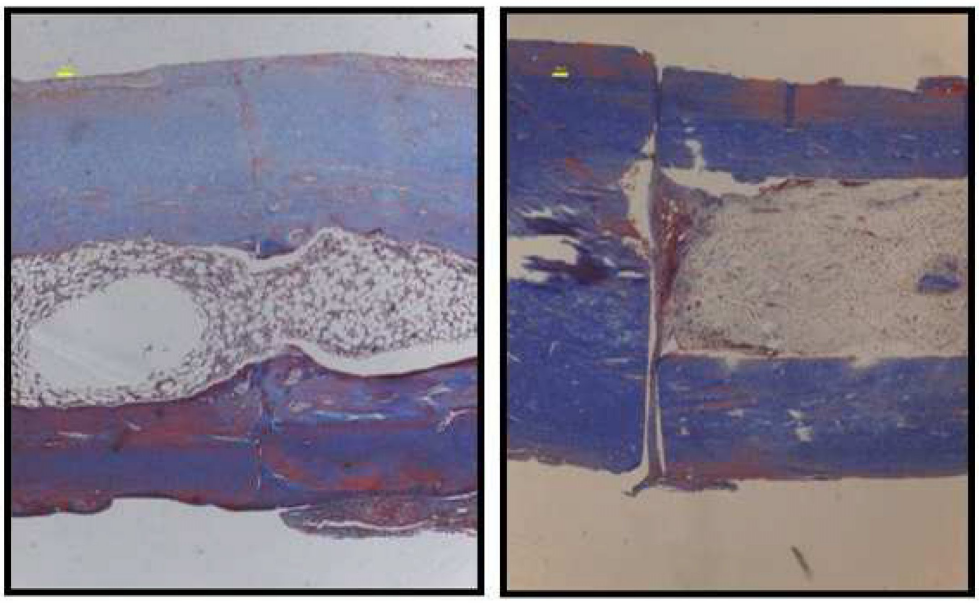

Figure 3. Inhibition of healing of an osteotomy by bisphosphonates (right panel) compared with normal healing of the osteotomy (left panel)

The mechanical environment is known to be of key importance in fracture repair. In particular, insufficient stability (eg to shear) can result in hypertrophic non-union. In contrast, locking plates, if used in a spanning fashion to heal with callus, often provide too much rigidity. Kenwright and Goodship[23] demonstrated that too much rigidity prevents healing and that ≈1 mm of movement at the fracture site improves healing. The movement that occurs at the fracture site is dependent on two factors: the stiffness of the construct and the amount of load applied.

For locking plates, the main determinant of the construct stiffness is the working length (ie the distance between the closest screws to the fracture site). However, this length is reduced if the plate is in contact with the cortex. Addition of more screws can reduce the risk of 'cut out' of the screws but does not change the stiffness greatly.

The amount that patients can weight bear varies widely. Even at 3 weeks, almost all of the patients could apply a load of <20 kg and even at 7 weeks 25% of patients could apply <20 kg.[24]

After consideration of these two parameters, the amount of interfragmentary movement can be modelled.[25] This model predicts that, because patients in the early stages are barely putting 200 N through the limb, to obtain a suitable amount of micromovement that is conducive with secondary healing the plate would need to have a working length of >70 mm. Thus, patients with a working length of <30 mm are at high risk of progression to non-union.

Thus, new technologies (whether they are pharmacological or implant devices) might have an inhibitory effect on fracture repair and give rise to new causes of non-union.

Finally, having discussed a range of points that we can optimise to maximise the chance of successful healing, I consider that there will still be some patients who are destined to develop non-unions. To help these patients, we must identify them at an early stage so that we can institute treatment to prevent non-union, such as stem-cell

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therapy.[26] The US Food and Drug Administration defines a non-union as a fracture that is ≥9 months old and has not shown signs of healing for 3 consecutive months. If a patient has reached this stage, we should consider that we have failed him/her and we should strive to make established non-union a 'disease of the past'.

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The Royal College of Surgeons of Edinburgh,
Nicolson Street, Edinburgh EH8 9DW
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