Research Report 2016–2018

THE ROYAL COLLEGE OF SURGEONS OF EDINBURGH

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Foreword from the President

One of the most important investments the College makes each year is in the next generation of surgical researchers. Supporting high-quality surgical research is essential if we are to make progress in our quest to provide better treatment for our patients. The following pages demonstrate our ongoing commitment to this vital field of research. From small bursaries to major partnerships, we are committed to delivering results that will, ultimately, improve and save lives.

This Research Report provides examples of the projects we have supported from a cross-section of medical and surgical disciplines. From our successful partnership with Royal Blind and innovations in ophthalmological care to our Clinical Training Fellowships with partners such as the Robertson Trust, the breadth of research and innovations is impressive and full of promise. We are looking constantly at different ways to support this important (but expensive) work.

A key part of our research strategy has been to embed research as part of training. Research is not an abstract exercise but a core part of making surgeons more curious and engaged with the conditions they are endeavouring to treat. Creating the space and time for research in a busy clinical career is always a challenge. Our College plays a vital part in encouraging surgeons in training to see the value of research and in enabling them to develop their clinical research skills by providing the necessary funding.

None of the extraordinary work in the following pages would be possible without the generous support of our donors. Trusts, foundations, partners and individuals have a crucial role in making our surgical research programmes viable. 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. I would also like to thank our dedicated Research Committee, which is chaired by Professor Stephen Wigmore. Their work is undertaken in a voluntary capacity and provides essential expertise and guidance. Without them, we could not deliver the multiple projects described in this report.

The future of our research programmes is exciting. Partnership working is allowing us to undertake more ambitious work that will have a national impact in the longer term. We are now looking to work with major medical research charities to grow surgical research from the periphery and place it at the heart of medical research agendas nationwide. This innovative approach to working together is in all of our interests and will leave a lasting legacy of treatments that will transform lives.

Professor Michael Lavelle-Jones
President of the Royal College of Surgeons of Edinburgh

Introduction from the Chairman

Supporting research and innovation in surgery is one of the core activities that the College provides to its membership. The Research Committee assesses and awards grants in several key areas, including cancer and non-cancer research, dental surgery, orthopaedic surgery, urology and vasculitis. We are grateful for the continued support and partnership of Royal Blind, which funds ophthalmology grants administered through the Ophthalmology Sub-Committee of the College. We look forward to developing new initiatives with them over the coming years. The Ophthalmology Sub-Committee is delighted to welcome Mr Mark O'Donnell, Chief Executive Officer of Royal Blind.

Many of the endowments that support the research activities of the College were established a considerable time ago and inflation of salaries and research costs has outstripped the increase in income revenue from investments. Therefore, to fund the Members and Fellows who apply for grants appropriately we must compromise by offering some of our Fellowships less frequently than in the past. The Research Committee believes that this is an important time to look at novel partnerships and new models of funding to try to reinvigorate the College's research profile. To this end, we are actively pursuing the creation of some new Fellowship funding opportunities, which we hope to announce over the next two years.

In the meantime, our Members and Fellows who are supported through existing funds continue to demonstrate excellence by converting pump-priming small-project grants into major Research Council funding and publishing impressive and impactful studies in high-ranking journals.

I would like to take this opportunity to thank the many partners, trusts and individuals who generously donate to our work. Their vision and commitment to this crucial area of medical research is deeply appreciated by the Research Committee and the College. I would also like to extend my thanks to Mrs Cathy McCartney who coordinates our grants and makes publications like this possible.

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Like many others, Research Committee members were saddened by the news regarding Professor Ken Fearon, who chaired our Committee for many years and who died tragically not long after the publication of the last Research Report.

Professor Stephen J Wigmore
Chair of the Research Committee

Donors to the RCSEd Research Fellowships, Grants, Bursaries

Alastair F Jamieson
Family of Alban Barros D'SA
Cutner Memorial Bequest Fund
Ethicon
Lindsay Stewart
Lorna Smith Charitable Trust Research Fellowship
Medical Research Council
Maurice Wohl Foundation
Mr Iain Fraser
Mr John Steyn and Family
Palliation and the Caring Hospital (Patch)
Robertson Trust
Royal Blind
Scottish Oral and Maxillofacial Society
Shanghai Head and Neck Maxillofacial Oncology Centre at the Ninth People's Hospital of Jiaotong University
Somes Guha
Wong Choon Hee Bursary

The College and the Research Committee gratefully acknowledge the donations from numerous Fellows of the College around the world.

Research Funding

Fellowships

- Joint RCSEd/MRC
- The Robertson Trust
- The Maurice Wohl
- Alastair F Jamieson
- Joint RCSEd/Cutner Fellowship in Orthopaedics
- Lorna Smith Charitable Trust Research Fellowship
- Joint RCSEd/SOMS/Shanghai Head and Neck Fellowship

Travelling Fellowships

- Cutner Travelling Fellowship in Orthopaedics
- Sir James Fraser Travelling Fellowship in General Surgery

Travelling Grants

- Ethicon Travel Grant

Bursaries

- Africa
- Undergraduate Elective or Vacation Studies
- RCSEd & Binks
- Wong Choon Hee

Medals

- Syme
- Dundas

Professorships

- King James IV Professorship
- Sushruta Professorship in Plastic Surgery

Research Grants

- Pump Priming Grants
- Ophthalmology Grants
- FST/ASME Grant
- Dental Educational Grant

Fellowship Awards

THE ROBERTSON TRUST RESEARCH FELLOWSHIP

Mr Robert Silverwood, Clinical Fellow, Trauma and Orthopaedics, University of Glasgow

"Development of a human three-dimensional osteoporotic model for the assessment of microRNA manipulation"

Due to ageing populations, osteoporosis represents a burden of ever-increasing proportions to healthcare systems worldwide. Current treatments have proven ineffective in stemming the tide of fragility fractures, and they are also associated with serious side effects. MicroRNAs are known to control gene expression in musculoskeletal diseases (e.g., osteoporosis) and represent a target for developing new treatments. I propose to undertake an assessment and subsequent manipulation of key microRNAs using a novel three-dimensional model of human osteoporosis. The results from this Fellowship project will increase the scientific knowledge of osteoporosis, and could contribute to the development of novel therapies.

(£50,000)

THE MAURICE WOHL RESEARCH FELLOWSHIP IN SURGERY AND DENTAL SURGERY

Miss Janice Miller, General Surgery Registrar/ Research Fellow in Clinical Surgery, Royal Infirmary of Edinburgh

"Muscle-wasting in cancer: molecular mechanisms and potential therapeutic targets"

Cachexia involves weight loss, fatigue, weakness and significant loss of appetite in someone who is not trying to lose weight actively. It cannot be reversed by conventional nutritional support and leads to progressive functional impairment. Cachexia is a major concern for cancer patients because it can lead to poor treatment outcomes after surgery and chemotherapy, as well as reduced quality of life and risk to survival.

We are investigating the mechanisms of how patients lose muscle and fat. Patients with cancers of the gullet, stomach and pancreas undergoing potentially curative surgery are recruited. Samples of blood, urine, fat and muscle are taken intraoperatively. The same samples are taken from patients undergoing non-cancer surgical procedures to allow comparisons. A better understanding of the pathways involved in tissue loss could lead to better avenues for treatment and overall improved survival.

(£50,000)

THE MEDICAL RESEARCH COUNCIL (MRC)/ROYAL COLLEGE OF SURGEONS OF EDINBURGH CLINICAL RESEARCH FELLOWSHIP

Mr Iestyn Shapey, Clinical Research Fellow, University of Manchester

"Insulin therapy in pancreas and islet transplantation"

In patients with complex type-1 diabetes mellitus (T1DM), pancreas and pancreatic-islet transplantation can be highly effective life-saving therapies. Two out of three organs offered for transplantation are considered unsuitable (perhaps inappropriately), and three out of 10 transplants will have failed by 5 years. Hence, there is an urgent need to: (a) improve methods for selecting good-quality donors; (b) identify the 'ideal' environment in which a transplanted pancreas can thrive.

Hyperglycaemia in organ donors in intensive care is common and is usually managed with insulin. Using the Maurice Wohl RCSEd Research Fellowship, I have shown that insulin use by a donor in intensive care results in a much higher prevalence of early transplant failure and worse function in solid-pancreas and pancreatic-islet-cell transplantation. These findings could suggest that insulin use by the donor is a sign that the donor pancreas is damaged, or that the cells are dying, which would make it less suitable for transplantation. I plan to use information on up to 50,000 recipients of a pancreas transplant to discover if insulin use by the donor helps identify organs that fail early. I will then analyse blood samples from pancreas donors and measure DNA which leaks into the blood when pancreatic beta cells die to ascertain if high blood sugar levels occur due to the stress or death of pancreatic beta cells.

In recipients of transplanted pancreatic islet cells, peri-transplant insulin therapy (which aims to normalise peri-transplant glucose levels) is the standard of care. However, this is not the case for solid-pancreas transplants and could be detrimental to the transplanted organs. I will, therefore, assess if good peri-transplant glycaemic control in recipients of pancreatic-islet transplants is associated with better function of pancreatic islets at 3 months. Then, using the evidence from my work as well the literature, patient/expert opinion,

and a local feasibility study, I will determine the evidence base and justification for a randomised controlled trial of peri-transplant insulin therapy in solid-pancreas transplantation to improve clinical outcomes.
(£102,384)

THE JOINT ROYAL COLLEGE OF SURGEONS OF EDINBURGH/CUTNER RESEARCH FELLOWSHIP IN ORTHOPAEDICS

Mr Anthony Sorial, NIHR Academic Clinical Fellow in Trauma and Orthopaedic Surgery, Institute of Genetic Medicine, Newcastle University

"Molecular genetics of osteoarthritis: enhancing cartilage integrity for future benefit to patients"
Inherited changes in our DNA (genes) affect the way our bodies are made and maintained. I seek to examine the genetic changes that cause osteoarthritis, a common painful disease of joints that affects older people usually and which can be difficult to treat, often necessitating surgery. I aim to use genome-editing methods to better determine which genes may be linked to an increased risk of osteoarthritis and if this risk can be altered. In future we hope to build on this research to engineer stronger cartilage in the laboratory with a view to developing new treatments for osteoarthritis.
(£60,000)

THE LORNA SMITH CHARITABLE TRUST RESEARCH FELLOWSHIP

Dan Pugh, Specialist Trainee in Nephrology, Centre for Cardiovascular Science, University of Edinburgh

"Chorioretinal thinning as a marker of disease activity in antineutrophil cytoplasmic antibody vasculitis"
My research will focus on using the eye as a 'window' to assess disease activity and the response to treatment in people with vasculitis. By using a new type of technology, 'optical coherence tomography', I aim to look in detail at the small blood vessels at the back of the eye. These vessels seem to mimic the activity of small blood vessels in the kidney. In doing so it may be possible to discover information about disease activity and response to treatment in people with vasculitis without the need for a kidney biopsy.
(£58,363)

Travelling Fellowship Awards

CUTNER TRAVELLING FELLOWSHIP IN ORTHOPAEDICS

Mr Andrew Monk
Specialist Training Registrar Year 8 (ST8) in Orthopaedics/National Institute for Health Research (NIHR) Academic Clinical Lecturer (ACL), Royal Berkshire Hospital, visiting Adidas UniSports Centre, University of Auckland
To study surgical interventions for early arthritis in the knee.
(£3,000)

Simon Graham
ST8, Orthopaedics and Trauma, Mersey Deanery. Orthopaedic Trauma and Research Fellowship, visiting Groote Schuur Hospital, University of Cape Town
(£1,500)

Muhammed Choudhury
Spinal Fellow, Scottish National Deformity Service, Royal Hospital for Sick Children, Edinburgh
To study anterior vertebral body tethering in the treatment of scoliosis, Shriners Hospital, Philadelphia.
(£1,500)

THE JOHN STEYN TRAVELLING FELLOWSHIP IN UROLOGY

Mr James Donaldson
ST7 in Urology, Western General Hospital, Edinburgh Renal Fellowship (laparoscopic and robotic), visiting Princess Alexandra Hospital, Brisbane
(£900)

ALBAN BARROS D'SA MEMORIAL TRAVELLING FELLOWSHIP IN GENERAL SURGERY

Mr Andrew Healey
ST8, Hepato-Pancreatico-Biliary (HPB) Surgery, Hammersmith Hospital, Imperial College Healthcare NHS Trust, visiting a Level 1 Tertiary University Trauma Centre, South Africa
The incidence of penetrating trauma in the UK is increasing. Arresting haemorrhage is the essential skill required of a trauma or resuscitative surgeon, and has traditionally been part of subspecialty vascular training. With the introduction of the new endovascular curriculum in 2013, there will be an increasing reliance on general or visceral surgeons to support the new major trauma centres in Scotland. I will assess how specialist trauma and liver surgeons in Groote Schuur Hospital, Cape Town, gain their surgical competencies in mutually exclusive training systems and how they share their expertise in the management of complex visceral polytrauma.
(£1,000)

Claire Rutherford
ST4 General Surgery, Queen Elizabeth University Hospital, Glasgow Breast Reconstruction Fellowship and Outreach, visiting KK Women's and Children's Hospital, Singapore
(£1,000)

THE SIR JAMES FRASER TRAVELLING FELLOWSHIP IN GENERAL SURGERY

Mr Mohan Singh
Specialist Training Registrar (SpR) in General Surgery, Royal Shrewsbury Hospital, Shrewsbury
I will visit the Department of Surgery at Queen Mary Hospital in Hong Kong (spearheaded by Professor Simon Law). This is renowned as the largest tertiary referral centre for gullet and stomach (oesophagogastric) cancers within the Asia-Pacific region. Their complex resections are performed with keyhole surgery and have exemplary long-term survival data. They have one of the lowest morbidity and mortality rates after gullet cancer surgery in the world. My learning objectives are: (i) minimally invasive surgery for oesophagogastric cancer with radical clearance of lymph nodes; (ii) endoscopic excisions of early cancers; (iii) observe effective delivery of an enhanced recovery programme after major surgery for cancer.
(£2,000)

Adam Frampton
ST6, General and Hepato-Pancreatico-Biliary and HPB Surgical Unit, Imperial College, London
To observe HPB surgery and transplantation at the Surgical Centre of Heidelberg University Hospital and European Pancreas Center in Germany.
(£2,000)

JOINT RCSED/SOMS/SHANGHAI HEAD AND NECK FELLOWSHIP

Owais Khattak
T6 Oral and Maxillofacial Surgery, Royal Blackburn Hospital, Blackburn Fellowship in Head and Neck Oncology at the Department of Cranio-Maxillofacial Science, School of Stomatology, Ninth People's Hospital, Shanghai Jiaotong University
(£3,000)

Mr Shofiq Islam
Specialist Registrar in Oral and Maxillofacial Surgery, East Midlands Fellowship in Head and Neck Oncology at the Department of Cranio-Maxillofacial Science, School of Stomatology, Ninth People's Hospital, Shanghai Jiaotong University
(£3,000)

Lindsay Stewart Awards

Michael Mwatsuma Mwachiro
Tenwek Hospital, Bomet, Kenya

"Determining the prevalence of oesophageal squamous dysplasia via Lugol's chromoendoscopy in south-western Kenya"
Dr Michael Mwachiro is a general surgeon at Tenwek Hospital, Bomet, Kenya. It is a training site for the Pan-African Academy of Christian Surgeons/College of Surgeons of East, Central and Southern Africa and a referral facility located in an area with a high prevalence of oesophageal cancer. Dr Mwachiro has active research interests in oesophageal squamous cell carcinoma (OSCC) and has been involved in multiple projects on its palliation, epidemiology and early detection.

Dr Mwachiro's project was a collaboration between Tenwek Hospital, the US National Cancer Institute (NCI), Mayo Cinic, and Department of Pathology of the University of Nairobi with funding from the African Organization for Research and Training in Africa and NCI Center for Global Health. The study aimed to determine the prevalence of oesophageal squamous dysplasia (a precursor lesion of oesophageal cancer) in asymptomatic adult residents. Individuals underwent a video-endoscopic procedure, staining with Lugol's iodine and biopsy of unstained lesions. They also answered a detailed questionnaire on risk factors and demographic data. Results were stratified by age, sex and geographic area of residence. A total of 305 people were enrolled and the total prevalence of dysplasia was 14.4%, with a prevalence of high-grade dysplasia of 2.9%. Age and geographic location were shown to have a significant association with dysplasia. This study has been published in the *American Journal of Gastroenterology* and demonstrated endoscopic screening to be feasible, safe and patient compliant in this high-risk population. This project on endoscopic screening with Lugol's chromoendoscopy is the first of its kind to be done in Africa, and could provide useful information for future screening projects for OSCC.
(£500)

Lydia Nanjula
Mulago National Referral Hospital, Uganda

"Competence-based training: the road to surgical proficiency based on the herniorrhapy prototype"
I set out to establish the relationship between competence-based training and proficiency using mesh repair of an inguinal hernia as a prototype. Nine trainees and three trainers were involved in a before-and-after evaluation of competence in undertaking the 39 steps of mesh repair of an inguinal hernia. The ability to carry out this procedure independently was the primary outcome measure and was achieved by 67% of trainees. Other outcomes included identification of inguinal nerves, tissue handling, use of diathermy, communication skills, and administration of local anaesthesia. I found a significant positive relationship between competence-based training and proficiency in mesh repair of an inguinal hernia.
(£500)

Ethicon Travel Grants

Saheel Mukhtar
Sindh Institute of Urology
and Transplantation, Pakistan
To gain further experience in urological stone disease.
(£1,000)

Paul Sutton
Division of Endoscopic Submucosal Dissection,
Japan, and the Richard Wolf Centre & Stadtisches
Kilnikum Karlsruhe Hospital, Germany
To study endoscopic submucosal dissection
and transanal endoscopic microsurgery.
(£870)

Louisa Ferguson
Otolaryngology Department at the
Royal Children's Hospital, Melbourne
Paediatric Otolaryngology Fellowship
(£800)

Simon Graham
Groote Schuur Hospital, University of Cape Town
Orthopaedic Trauma Fellowship
(£1,000)

Robert Choa
Fiona Stanley Hospital, Perth, Australia
Microsurgery, Burns and Skin Oncology Fellowship
(£1,000)

Ophthalmology Awards

FUNDED BY ROYAL BLIND SMALL PROJECT GRANTS

Hari Jayaram
UCL Institute of Ophthalmology, London
"Implementation of the controlled elevation of intraocular pressure (CEIP) model of experimental rodent glaucoma in the United Kingdom and investigation of the impact of CEIP exposure upon organisation of retinal ganglion cells within the retina"
(£10,000)

MAJOR PROJECT GRANTS

David Charteris, Tennent Institute of Ophthalmology, Gartnavel Hospital, Glasgow
"A randomised controlled trial to evaluate the effect of face-down posturing on retinal displacement and distortion following retinal-detachment repair"
(£60,000)

Robert MacLaren, Nuffield Laboratory of Ophthalmology, University of Oxford
"Development of a CRISPR gene-therapy system for treating inherited retinal degenerations"
(£59,228)

Mandeep Sagoo, UCL Institute of Ophthalmology, London
"New mechanisms and treatment targets in retinoblastoma"
(£30,000)

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

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 Alexandra 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)

Robert MacLaren, Nuffield Laboratory of Ophthalmology, University of Oxford
"Optimising gene therapy to improve patient safety"
(£49,971)

King James IV Professorship Awards

Mr Alastair Gibson, Consultant Orthopaedic Spinal Surgeon, the Royal Infirmary of Edinburgh, Honorary Senior Lecturer, University of Edinburgh
"Advances in endoscopic spinal surgery and application of new techniques for the treatment of cervical-disc prolapse"
(£500)

Dr Mandeep Sagoo, Moorfields Eye Hospital, Saint Bartholomew's and the Royal London Hospitals, UCL Institute of Ophthalmology, London
"Multimodality treatment of eye tumours: advances in chemotherapy, radiotherapy and surgery"
(£500)

Mr Ian Ormiston, Consultant Oral and Maxillofacial Surgeon, Leicester Royal Infirmary NHS Trust
"Expanded horizons for maxillofacial orthognathic surgical techniques: upper-airway manipulation and its applications in treating obstructive sleep apnoea"
(£500)

Jon Clasper, Consultant Orthopaedic Surgeon, Frimley Park Hospital Foundation Trust
"New insights into the mechanisms of injury from blasts"
(£500)

Fergal Monsell, Consultant Paediatric Orthopaedic Surgeon, The Children's Hospital for Wales, Cardiff
"Some observations on limb reconstruction in children: where are we, how did we get here and where are we going?"
(£500)

Sushruta-Guha Professorship Award

Dr Pohdan Pomahac, Plastic Surgeon,
Director of Plastic Surgery, Transplantation,
Brigham & Women's Hospital, Boston, Massachusetts
"Facial restoration by transplantation"
($3,500)

Syme Medal Awards

Mr Iain Murray, Edinburgh Clinical Academic Track (ECAT) Clinical Lecturer, Department of Orthopaedic Surgery, University of Edinburgh
"Establishing a critical role for AV integrins on perivascular mesenchymal cells in regulating skeletal and cardiac muscle fibrosis"

Mr Chris Johnston, Clinical Lecturer/Honorary Specialty Registrar in General Surgery, University of Edinburgh/NHS Lothian
"Exploration of helminth-derived immunoregulatory molecules as options for therapeutic intervention in allograft rejection"

Mr Benjamin Dean, Doctoral Student, University of Oxford
"Role of glutamate in rotator cuff tendinopathy"

Mr Neil Johns, SpR in Surgery, University of Edinburgh
"Skeletal muscle wasting and oncological outcomes"

Mr Benjamin Stutchfield, Clinical Lecturer and Honorary Surgical Registrar, University of Edinburgh
"Supporting the failing liver"

Nicholas Ventham, Speciality Training in General Surgery, University of Edinburgh
"Integrative epigenome-wide analysis demonstrates that DNA methylation may mediate genetic risk in inflammatory bowel disease"

Aidan Rose, SCREDS Clinical Lecturer in Plastic Surgery, Department of Cancer Research, School of Medicine, University of Dundee
"Role of transforming growth factor-beta signalling in human cutaneous squamous cell carcinoma"

Mary Eastwood, Trust Fellow in Paediatric Surgical Specialties, Great Ormond Street, London
"Perinatal solutions for congenital diaphragmatic hernia"

Iain Nixon, Consultant ENT Surgeon, NHS Lothian, Edinburgh
"Impact of my research on management of differentiated thyroid cancer"

Dundas Medal Award

Dr Alistair McKeown, Consultant, the Prince and Princess of Wales Hospice, Glasgow, and Queen Elizabeth University Hospital Specialist Palliative Care Team, Glasgow

Small Pump-Priming Grants

Miss Rachael Jablonski, Academic Clinical Fellow in Restorative Dentistry, Leeds Dental Institute
"Proof-of-concept study to assess the feasibility and accuracy of capturing facial defects based on surface scanning of plaster casts"
(£9,998)

Ms Suzanne Thomson, MRC Clinical Research Fellow, University of Glasgow
"Investigation of strategies to enable objective quantification of neural regeneration through tissue-engineering conduits *in vivo*: preparing for clinical trials"
(£9,886)

Miss Victoria Banwell, Clinical Research Fellow in Transplantation Surgery, MRC Centre for Inflammation Research, University of Edinburgh
"Role of microRNA-214 in the pathogenesis of chronic renal allograft damage"
(£10,000)

Mr Siong-Seng Liau, MRC/Academy of Medical Sciences Tenure-track Clinical Scientist, Honorary Consultant in Hepatobiliary and Pancreatic Surgery, University of Cambridge
"Utilising a novel *in vitro* model of partner and localiser of BRCA2 (PLALB2)-associated pancreatic adenocarcinoma to understand the genomic landscape of DNA repair-deficient tumours"
(£10,000)

Mr David Metcalfe, Clinical Research Fellow in Musculoskeletal Trauma, University of Oxford
"Total hip replacement versus hemiarthroplasty for independently mobile older adults with an intracapsular hip fracture"
£5,400)

Dr Adarsh Kudva, Assistant Professor, Manipal College of Dental Sciences, Manipal University, Dubai
"Salivary DNA methylation markers for early detection of oral squamous cell carcinomas"
(£10,000)

Mr Adam Frampton, Honorary Clinical Lecturer in Surgery and Cancer, Imperial College London
"Integrated analysis of non-coding RNA markers for detecting and stratifying pancreatic cancer"
(£9,938)

Dr Richard McGregor, Clinical Lecturer in General Surgery, University of Edinburgh
"Identification of WILM's tumour-1-expressing cells in adult angiogenesis"
(£9,604)

Mr Zahid Khan, Specialty Dentist in Oral Medicine and Clinical Lecturer, Birmingham Dental Hospital and School of Dentistry
"Molecular and cellular characterisation of oral lichen planus"
(£8,077)

Miss Marie Kearns, Clinical Research Fellow, Canniesburn Plastic Surgery Unit, Glasgow
"Adipose-derived stem cells to promote regeneration after peripheral nerve injury"
(£7,902)

Mr Steve Leung, Consultant Urologist, Urology Department, Western General Hospital, Edinburgh
"The diagnostic value of urinary cytology in men with a suspected diagnosis of prostate cancer"
(£8,783)

Mr Robert Silverwood, Clinical Fellow, Queen Elizabeth University Hospital, Glasgow
"Development of a human three-dimensional osteoporotic model for the assessment of microRNA manipulation"
(£9,468)

Mr Paul Sutton, Specialty Registrar/Honorary Senior Lecturer, University of Liverpool
"Does inhibiting acid ceramidase improve radiosensitivity in an *in vitro* model of colorectal cancer?"
(£8,900)

Mr Anantha Madhavan, Registrar in General Surgery, Newcastle University
"Does the transcriptome of disseminated tumour cells in blood and bone marrow explain or predict early recurrence of patients with oesophageal adenocarcinoma?"
(£9,997)

Mark Hughes, ECAT Clinical Lecturer, Centre for Integrative Physiology, University of Edinburgh
"Exploring the effect of nanoscale mechano-transduction on human glioblastoma stem-like cell differentiation"
(£10,000)

Adam Frampton, Honorary Clinical Lecturer in Surgery and Cancer, Department of Surgery and Cancer, Imperial College London
"Endoscopic molecular markers for detecting pancreatic malignant transformation"
(£9,973)

Rachael Forsyth, Honorary Research Fellow, Centre for Cardiovascular Science, University of Edinburgh
"Edin-Vasc molecular imaging study"
(£9,986)

Richard Taylor, Clinical Research Fellow, Division of Cancer Research, University of Dundee
"Mechanism of action of pro-tumourigenic transforming growth factor-beta signalling in head and neck squamous cell carcinoma"
(£9,998)

Li Yong, Clinical Research Fellow, MRC Centre for Regenerative Medicine, University of Edinburgh
"Tissue-specific stem cells and tissue-matched hydrogels; the natural answer for auricular tissue engineering"
(£10,000)

John Kennedy, Orthopaedic Registrar, Centre for Cellular Engineering, University of Glasgow
"Nanoscale vibrations to modulate osteogenesis"
(£10,000)

Janice Miller, SpR in General Surgery, Clinical Surgery, University of Edinburgh
"Adipose depot gene expression in cancer cachexia"
(£9,435)

Mr David Green, Specialty Registrar (StR) in Restorative Dentistry/Honorary Clinical Lecturer, University of Birmingham School of Dentistry
"Utilising bioactive molecules in a dentine matrix for regeneration of the dental pulp following disease"
(£9,750)

Mr John O'Neill, Clinical Lecturer, Cancer Research UK, Edinburgh Centre, Edinburgh
"Characterising the cellular and transcriptional features of lymphatic metastasis in oesophageal adenocarcinoma"
(£10,000)

Mr Innes Smith, Clinical Lecturer in Orthopaedics, Institute of Infection, Immunity and Inflammation, University of Glasgow
"Whole-blood transcriptomic analysis in children presenting with acute monoarthritis: identification of gene expression biomarkers unique to septic arthritis"
(£9,681)

Ms Katie Connor, Research Fellow in Transplantation Surgery, the Queen's Medical Research Institute, University of Edinburgh
"MicroRNAs regulate the heme-oxygenase-1 response to heme arginate in renal transplant recipients"
(£10,000)

Mr Richard Taylor, Clinical Research Fellow, Jacqui Wood Cancer Centre, Division of Cancer Research, Ninewells Medical School, University of Dundee
"Deciphering the transforming growth factor-beta paradox in head and neck squamous cell carcinoma using next-generation transcriptome sequencing"
(£9,995)

DENTAL EDUCATIONAL GRANTS

Ms Hannah Crane, Academic Unit of Oral and Maxillofacial Pathology, the School of Clinical Dentistry, Sheffield Edge Hill University
PGCert Teaching and Learning in Clinical Practice

Mr Mohammed Dungarwalla, Queen Victoria Hospital, East Grinstead
Postgraduate Certificate in Medical Education, Newcastle University

Faculty of Surgical Trainers/Association for the Study of Medical Education (FST/ASME) Educational Research Grants

Paul Sutton, StR and Honorary Senior Lecturer, University of Liverpool

"Exploring clinical decision-making among surgical trainees in a simulated environment"
Clinical decision-making is a relatively poorly understood non-technical skill, but one that is essential to surgeons in and out of the operating theatre. We have planned a pilot study to help better understand clinical decision-making in an acute clinical setting: assessment and management of the critically ill surgical patient. We utilise a simulated scenario after which the participant watches the video with the investigator and the performance is evaluated using teach-back interviewing. The transcripts of these interviews will be analysed thematically using standardised methods to explore behaviourism with respect to decision-making.
(£1,920)

Sotiris Papaspyros, ST7 Cardiothoracic trainee, Royal Infirmary of Edinburgh

"Reliability of low-fidelity simulation models in acquisition of basic surgical skills outside the operating room. The role of deliberate practice"
Surgical training has evolved to conform with several limitations: a shorter working week for residents, an increasing complexity of cases, emphasis on efficiency in the operating room and a mitigation of medical errors. Acquisition of basic surgical skills can occur outside the operating room on low-fidelity, readily available simulation materials (e.g., bananas, potatoes, poached eggs). Deliberate practice can provide the educational framework to achieve competence in surgical tasks (e.g., needle rotation, economy of movement, pace). We aim to provide evidence that low-fidelity simulation models and deliberate practice can, reliably and consistently, be used to teach novice aspiring surgeons basic surgical skills outside the operating room.
(£1,200)

Joshil Lodhia, Cardiothoracic Trainee, Leeds General Infirmary

"Quantitative motion analysis of surgical skills to assess improvement in trainees' performance following deliberate practice"
Surgical training in previous decades was dependent on the speed of operating and obtaining a high volume of cases. Due to the European Working Time Directive and the need to ensure the highest standards, trainees can no longer obtain this volume of cases. Training in the art of surgery must become more explicit. We aim to assess the fine movements of surgery with the use of magnetic sensors. This strategy will allow trainers and trainees to ensure that the subtleties of surgery can be developed in a safe environment outside operating theatres before ensuring a high level of skill.
(£2,000)

Student Bursary Awards

AFRICA BURSARIES

D Barkwill, University of East Anglia
Visiting Chidamoyo Hospital, Zimbabwe
(£500)

S Tingle, Newcastle University
Visiting Haydom Lutheran Hospital, Tanzania
(£500)

P Chauhan, University of Birmingham
Visiting the Department of General Surgery,
Kilimanjaro Christian Medical Centre, Tanzania
(£500)

V Naruka, University of Cambridge
Visiting Somerset Hospital, Cape Town
(£500)

Miss Helen Please, University of Oxford
Visiting the Good News Hospital, Madagascar
(£500)

Mr James Bruce, University of Bristol
Visiting the AMREF Flying Doctors,
and Mnazi Mmoji Hospital, Tanzania
(£500)

Mr Alexander North, University of Dundee
Visiting University of the Witswatersrand, South Africa
(£500)

Adam Tollitt, University College London
Visiting Urambo District Hospital, Tanzania
(£500)

Ethicon Bursaries

Yasser Al Omran, Barts and the London School of Medicine and Dentistry
Undertaking plastic surgery at New York Presbyterian Hospital
(£250)

John Allen, Imperial College London
Visiting the Department of Otolaryngology – Head and Neck Surgery at the Johns Hopkins Hospital, Baltimore
(£250)

Ryan Preece, Cardiff University
Visiting the Department of Surgery, Karapitiya Teaching Hospital, Sri Lanka
(£250)

Zaamin Hussain, University of Cambridge
Visiting an orthopaedic clinic in Vail, Colorado
(£250)

Catherine Lovegrove, King's College London
Visiting the Urology Department within the Roswell Park Cancer Institute, New York, and the Johns Hopkins Hospital, Baltimore
(£250)

Bursaries for Undergraduate Elective or Vacation Awards

Heather Leather, Centre for Global Health, King's College London
"A systematic review of the role of community health workers in surgical assessment within low- and middle-income settings"
(£469)

Marc Walton, Centre for Integrative Physiology, University of Edinburgh
"Implanted silicon systems for monitoring and targeted drug delivery in glioblastoma multiforme: assessing the bio-compatibility for candidate flexible electronic substrates"
(£1,500)

Ellhia Sudan, University of Edinburgh
"Characterisation of the microenvironment of hepatocellular carcinoma in non-alcoholic fatty liver disease with or without cirrhosis"
(£1,000)

Savva Pronin, Department of Clinical Neuroscience, Western General Hospital, Edinburgh
"Audit of management of cauda equine syndrome and MRI-negative cauda equine syndrome in NHS Lothian"
(£960)

Sonia Soopen, School of Dentistry, University of Birmingham
"An *in vitro* study of the ability of a tricalcium silicate-based endodontic sealer to produce an effective three-dimensional seal using the single-cone obturation method"
(£900)

Ananyo Bagchi, The Walton Centre NHS Foundation Trust, Liverpool
"Incidence of postoperative epilepsy in seizure-naïve patients undergoing craniotomy for resection of meningioma"
(£960)

Etienne Chew, Urology Department, Western General Hospital Edinburgh
"Renal function outcomes after radical and partial nephrectomy: a national collaborative study"
(£1,200)

Thineskrishna Anabarasan, Division of Cancer Research, Ninewells Hospital, Dundee
"Risk of complications and mortality following prostate biopsy: a retrospective longitudinal study"
(£1,200)

Tausif Huq, Department of Surgery and Cancer (Surgery), Imperial College London
"Direct-from-sample rapid evaporative ionisation mass spectromery as a screening tool for the early detection of colorectal cancer: a pilot prospective observational study"
(£1,500)

Wong Choon Hee Bursaries

Luke Chan, University of Edinburgh
Visiting the Urology Unit and Trauma Unit
at the Royal Melbourne Hospital, Australia
(£500)

Paul McLean, University of Dundee
Visiting the Department of Head and Neck Surgery,
Memorial Sloan Kettering Cancer Center, New York
(£250)

Susanne Flach, University of Oxford
Visiting the Department of ENT, Head and Neck Surgery,
Bern, Switzerland, and Department of Orthopaedics,
Western Regional Hospital, Nepal
(£250)

Adam Bhanji, Manchester University Medical School
Undertaking dental care and oral maxillofacial care
at Mercy Ship, Cameroon
(£500)

Gwyneth Jensen, Queen Mary University of London
Visiting the Department of Burns and Plastic Surgery at
Kirtipur Hospital and Lalgadh Leprosy Hospital, Nepal
(£250)

Jack Kingdon, King's College London
Visiting the Department of General/Trauma Surgery
at Tygerberg Hospital and Department of Orthopaedic
Surgery, Groote Schuur Hospital, South Africa
(£250)

Roxanne Tajbakhsh, University of Leeds
Visiting Universita del Piemonte Orientale, Novara, Italy,
and the Cook County Trauma and Burn Unit, Chicago
(£250)

Fellowship Report

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

Peter Young
Centre for Cell Engineering, University of Glasgow
Robertson Trust Grant
February 2016 to February 2017

LAY SUMMARY

Orthopaedic implants suffer from weak attachment to bone, and osteoporosis (loss of bone density) is a growing healthcare burden. My project aimed to develop implants that could help control stem-cell fate, increase the number of bone-forming cells (osteoblasts) and new bone while reducing the number of bone-resorbing cells (osteoclasts). We know that specific nanopatterns can increase bone formation. However, the early part of my research showed that osteoclasts do not respond to these nanopatterns. I collaborated with an engineering colleague to combine the potential of nanopatterning with strontium, an element closely related to calcium that has been shown to reduce the number of osteoclasts while increasing the number of osteoblasts.

We developed nanopatterned titanium surfaces that incorporated strontium. These surfaces increased new-bone formation dramatically, and did not allow osteoclasts to attach to surfaces because bone-forming cells produced proteins that opposed osteoclasts. Then, I used advanced genetic and metabolomic analyses to show that the cells responded to the surfaces as fractures requiring healing. We identified new targets which could be used for osteoporosis treatment.

These surfaces have great potential in the design of orthopaedic implants, and we have several potential targets to treat osteoporosis, which will require further research.

GRANT REPORT

(A) CLINICAL AND SCIENTIFIC SIGNIFICANCE OF ADVANCES MADE

Mesenchymal stem cells (MSCs) are multipotent cells that are highly responsive to their environment and give rise to cells of the stromal lineage, such as bone-forming osteoblasts. However, the MSCs present in bone marrow respond to traditional biomedical implants by production of soft tissue rather than bone, which can result in early failure, particularly of load-bearing prostheses. The failure mechanisms vary, but they are largely related to soft-tissue encapsulation or the immune response caused by arthroplasty-related particles, with infiltration of macrophages and osteoclasts resulting in osteolysis and eventual loosening. The burden of morbidity related to these failure mechanisms is set to increase as the prevalence of arthroplasty increases. Methods to improve implant integration, such as surface coating, have limited osseointegration, and the development of implants is dominated by the need to promote osteogenesis and osseointegration.

Varying the features of the material surface, such as its chemistry, stiffness and topography, alters the adhesion, proliferation and growth of cells. Recent evidence has shown that an optimised topography with nanoscale pillars arranged in a disordered (but controlled) manner (e.g., NSQ50) increases osteoblast differentiation from MSCs *in vitro* with a similar efficiency to standard chemical treatments. Use of nanotopography to promote cell adhesion and bone formation provides an excellent opportunity to produce implants requiring areas of osseointegration, but the response of osteoclasts is not known.

Osteoporosis involves a reduction in bone density that occurs with ageing, and can lead to fragility fractures, particularly around the spine and hip. These fractures are associated with significant morbidity and mortality, costing the National Health Service (NHS) £2 billion per year. Traditional treatments for osteoporosis, such as bisphosphonates, are antiresorptive, and halt further decline without improving bone formation. Strontium is similar to calcium and is used as an oral treatment for osteoporosis. Strontium is one of very few treatments that

is anabolic and increases bone density. However, in the oral form it has been shown to have off-target side effects on the heart and gut, leading to limited use despite a good clinical effect.

During my research I developed and evaluated custom-designed surfaces that combine the osteogenic synergy of nanopatterning and strontium. I established that the surfaces can be produced with high fidelity and can elute strontium up to a maximum cumulative dose of ≈800 ng/cm^2. About 600 ng is eluted rapidly within the first 24 h by Fickian diffusion, followed by a more gradual elution up to 35 days.

An interesting initial finding in the biological assessment of surfaces was the failure to establish a co-culture of bone-marrow stem cells (BMSCs) and bone-marrow haematopoietic stem cells (BMHSCs) on materials using a method established on polycarbonate. Further investigation showed that it is the response of the BMSCs to the titanium surfaces that modifies the BMHSC response. In isolation, macrophages attach well to the surfaces and persist but, with the addition of BMSCs (which produce high quantities of osteoprotegerin and no or very minimal macrophage colony-stimulating factor or receptor activator of nuclear factor kappa-B ligand), the macrophages undergo apoptosis.

On the surfaces, BMSCs attached well and produced a significantly increased extracellular matrix, greater amounts of the protein associated with bone formation (osteocalcin), and increased mineralisation compared with control surfaces. It appears that the strontium incorporated into the surface was more important in the cell response than the eluted strontium. I identified the likely genetic and metabolomic mechanisms by which the nanopatterned and strontium-incorporated surfaces appear to act. These are predominately linked with signalling by integrin and calcium channels. Overall, the gene and metabolomic changes caused by the surfaces bore significant similarities to the changes seen during fracture healing. This leads me to believe that the surfaces are detected as fracture calluses requiring mineralisation. Several gene-signalling 'hubs' and metabolites of interest were identified that may be used to reproduce the effects of the surfaces using chemical or drug treatments.

Overall, my research year enabled me to develop surfaces with great potential in orthopaedics, which we plan to take into *in vivo* trials. These surfaces, if applied to orthopaedic and dental implants, could improve osseointegration, elongate the lifespan of implants, and help patients avoid revision surgery with its inherent morbidity and mortality. Furthermore, genetic and metabolic analyses enabled me to identify potential biomarkers that merit further investigation in osteoporosis treatment.

(B) PROBLEMS ENCOUNTERED AND THE STEPS TAKEN TO OVERCOME THEM

Initially, my Fellowship grant was undertaken to look into the effects of nanoscale-patterned surfaces on osteoclast differentiation. However, it became evident early on in my research that osteoclasts and their macrophage precursors do not appear to respond to nanoscale topographical patterning in the same manner as MSCs. This observation led to the idea of combining surface chemistry with topography to optimise osteoblast differentiation using topography and to influence osteoclastic differentiation simultaneously using chemistry. Therefore, I began collaboration with the Department of Engineering at the University of Glasgow, which had the expertise on materials to produce titanium samples with nanopatterning and strontium incorporated into the surfaces. Serendipitously this has led to a much more rewarding project, with orthopaedically relevant surfaces that may optimise osseointegration, and which merit *in vivo* evaluation. Furthermore, these surfaces provide an excellent opportunity to develop new targets for osteoporosis treatment.

(C) COLLABORATIONS ESTABLISHED

The first collaboration was with Dr Andrew Greer and Professor Nikolaj Gadegaard in the Department of Engineering at the University of Glasgow, with whom we developed the surfaces. This collaboration will be continued into *in vivo* trials in the near future. Another collaboration was made with the Glasgow Polyomics Facility, where we undertook advanced next-generation sequencing and metabolic analyses.

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

I obtained a doctorate from the University of Glasgow based on this research in August 2017. I am now an Honorary Clinical Fellow at the University of Glasgow and am training as a problem-based learning facilitator. I was awarded a Wellcome Trust grant based on this research (105614/Z/14/Z). I have published one study and I am authoring a review article and a research paper based on this research for submission to a high-impact scientific journal *(ACS Nano)*.

Scientific articles

- Young PS, Tsimbouri MP, Gadegaard N, et al. Osteoclastogenesis/osteoblastogenesis using human bone marrow derived co-cultures on nanotopographical polymer surfaces. *Nanomedicine* 2015;10:949–957.

Oral presentations

- Young PS, Greer AIM, Tsimbouri MP, et al. Precision-engineered strontium-eluting nanopatterned surfaces to control bone formation. British Orthopaedic Research Society (BORS), 2016, Glasgow.
- Young PS, Greer AIM, Tsimbouri MP, et al. Strontium-eluting nanotopographical surfaces to control bone homeostasis. Glasgow Meeting of Orthopaedic Research, 2017, Glasgow [winner of the Ronald McRae prize for best oral presentation].
- Young PS, Greer AIM, Tsimbouri MP, et al. Strontium-eluting nanotopographical surfaces to control bone homeostasis. Accepted for presentation at the British Orthopaedic Association conference, November 2017, as the 'Best of the best section'.

(E) ACKNOWLEDGEMENTS

I acknowledge financial support from the Robertson Trust and Royal College of Surgeons of Edinburgh and the help of my supervisors Professor Matthew Dalby, Dr Monica Tsimbouri, Dr Carl Goodyear and Professor Nikolaj Gadegaard, without whom my research degree would not have been possible. I also acknowledge the help of my collaborator in producing the surfaces, Dr Andrew Greer, our laboratory technician, Carol Anne Smith, and the staff and researchers at the Centre for Cell Engineering and Glasgow Polyomics Facility. My final thanks go to my family for their support throughout.

Fellowship Report

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

Amir Awwad
Radiological Sciences, Sir Peter Mansfield Imaging Centre (SPMIC),
School of Medicine, Queen's Medical Centre, Nottingham University Hospitals NHS Trust
Alastair F Jamieson Fellowship
21 October 2015 to 26 October 2016

LAY SUMMARY

We wished to show that the haemodynamic forces of the aneurysm as assessed by advanced and non-invasive four-dimensional magnetic resonance angiography (4D-MRA) in patients with abdominal aortic aneurysms (AAAs) can be used to predict aneurysmal growth and, thus, rupture risk.

Using 4D-MRA we identified the haemodynamic imaging markers associated with aneurysmal growth. This was a key step towards the ulterior aim of improved individualised management of asymptomatic AAA based on the real-time biomechanical data. This pilot study was the start of a larger, confirmatory natural-history study.

Three main hypotheses were tested:

(i) Energy dissipation contributes to AAA growth and indicates rupture risk
(ii) Aortic stiffness contributes to AAA growth and indicates rupture risk
(iii)4D-MRA affords reliable assessment of core haemodynamic metrics, such as energy loss and aortic stiffness.

This was a prospective, cohort, longitudinal, observational, pilot study involving 75 patients with AAAs and 15 controls.

4D-MRA is a novel magnetic resonance imaging (MRI) sequence synchronised with electrocardiography (ECG) gating and respiratory compensatory gating to capture time-resolved, flow-sensitive imaging data. This is considered to be dynamic assessment of the clinically known precursor phase contrast-MRI but in real-time (in this context time is the added fourth dimension). 4D-MRA can detect the change in particle velocity and ultimately provide image contrast to demonstrate blood flow in vessels.

GRANT REPORT

(A) CLINICAL AND SCIENTIFIC SIGNIFICANCE OF ADVANCES MADE

- Fellowship time allowed for Out of Programme for Research (OOPR) pre-approved by the General Medical Council, Royal College of Radiologists and High Education East Midlands (HEEM) deanery.
- Ethical approval achieved, trust (site) sponsorship and other funding secured.
- On-target recruitment from local AAA surveillance and NHS screening programme.
- Approval for National Institute for Health Research (NIHR) Portfolio-adopted study (attracting other UK investigators for follow-up and offers of participation).
- Annual review by ethics committee and NIHR portfolio team was satisfactory.
- More robust scanning protocol used in research study (pre-clinical pilot scans).
- Cohort analysis (ongoing) of 8 years of AAA sonographic follow-up (registered audit).
- Enriched experience of phantom-flow MRI.
- Doctoral thesis submitted in December 2017.
- Manuscripts submitted to the *Journal of Magnetic Resonance Imaging:* awaiting reviewers' verdicts.

(B) PROBLEMS ENCOUNTERED AND STEPS TAKEN TO OVERCOME THEM

- Ethics application was achieved even though the content was highly technical.
- Vast amounts of imaging data were collected. This was anticipated so storage capacity was enhanced with encrypted data protection on big back-ups (local and remote).
- Some scan-related issues which were managed (e.g., initially ECG-gated imaging was problematic, then ECG leads were adjusted with use of arrhythmia windows).

(C) COLLABORATIONS ESTABLISHED

- Vascular and Endovascular Surgery Department (co-sponsor and funder)
- Biomedical Engineering, University of Zurich (Professor S Kozerke)
- Lucas Medical Imaging Centre, Stanford University (Professor M Alley)
- Amsterdam Medical Centre (MRI Facility for 4D MRI Phantom Imaging, Professor G Strijker)
- Vascular Flow Technologies (Dundee, Scotland)
- Bioengineering Department, University of Glasgow (Professor R Black)

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

- Obtained my doctorate from the University of Nottingham in December 2017.
- American Heart Association Award (North American Society for Cardiovascular Imaging, Baltimore, October 2016): published abstract.
- Roentgenfest First Research Winner (Anu Damera Prize, November 2016).
- Small grant "Vascular Research Fund", Nottingham (RC48CK; MRI scan costs).
- Small department funding to visit Amsterdam Medical Centre (July 2016).

(E) ACKNOWLEDGEMENTS

- Nottingham Hospital Charity for their support for doctoral tuition fees (RB48BK).
- University of Nottingham Business Park for assisting in MoU with VFT agreement .
- Alastair F Jamieson.

Fellowship Report

Role of DNA supercoiling in rheumatoid arthritis

Barbara Hauser
Rheumatic Disease Unit, Institute of Genetics and Molecular Medicine,
Western General Hospital Edinburgh, University of Edinburgh
Lorna Smith Charitable Trust Research Grant
1 October 2013 to 30 June 2015

LAY SUMMARY

Rheumatoid arthritis (RA) is a common form of arthritis characterised by chronic pain and swelling of (primarily) the small joints of the hands and feet, which can lead to joint destruction and debilitating deformities.

RA development is influenced (at least in part) by environmental factors (e.g., smoking) and genetic factors. However, little is known about the impact of epigenetic factors (i.e., elements within the cell), which 'fine tune' the reading of the genetic code (DNA) as RA triggers.

Our aim was to investigate: (i) the shape and twisting of the DNA ("DNA supercoiling") in human cells; (ii) if DNA supercoiling influences the production of autoantibodies (types of protein that attack parts of your body) and RA development.

Our research team focused on B and T lymphocytes, which are thought to be crucial players in RA development. We isolated these cells from patients and healthy controls. We established a novel protocol how to process these cells to allow visualisation and quantification of DNA supercoiling in human cells. We are now in a position to analyse specific genetic regions of interest using microarray technology.

GRANT REPORT

(A) CLINICAL AND SCIENTIFIC SIGNIFICANCE OF ADVANCES MADE

- We established a protocol for how to isolate cluster of differentiation (CD)3+ and CD2+T cells and CD19+ CD20+ B cells from whole blood. We isolated an average of 8×10^5 B cells/ml and 4×10^6 T cells/ml. Fluorescence-activated cell sorting (FACS) analyses confirmed the isolation of T and B cells with an average purity of 87% and 74%, respectively.
- We further developed a protocol for high-resolution mapping of DNA structure of B and T cells with biotinylated-trimethyl psoralen (bTMP) at 500 µg/ml and ultraviolet (UV) crosslinking. Briefly, T and B cells were treated with bTMP and incubated for 20 min. The cell suspension was UV-crosslinked and prepared for sonication. The presence of psoralen-bound DNA was confirmed with gel electrophoresis and dot blots. Sonicated chromatin samples were separated for chromatin immunoprecipitation (ChIP), which then allowed microarray analyses. This is the first research protocol of this type on human cells.
- After initial difficulties, we obtained ethical approval to obtain ≤100 ml of blood per donor, which is required to obtain sufficient numbers of B and T cells for molecular experiments.
- Through literature searching we identified several genomic regions of interest that can be investigated for DNA supercoiling: human leucocyte antigen; nuclear factor-kappa B; protein tyrosine phosphatase, non-receptor type 22; interleukin (IL)20; IL22; tumour necrosis factor-alpha-induced protein 3; C-C motif chemokine receptor 6; IL1 receptor family; IL1A; IL1B; IL6; IL6 receptor; IL17 receptor B; IL17A; IL17F; peptidyl arginine deiminase 4.

(B) PROBLEMS ENCOUNTERED AND STEPS TAKEN TO OVERCOME THEM

- Previously, ethical approval allowed a maximum of only 50 ml of blood per donor to be obtained. However, we discovered that to isolate a sufficient number of B and T cells, we required 100 ml of whole blood. We applied for an amendment of ethical approval to allow us to obtain the adequate amount of blood from patients and healthy controls. This amendment was approved.

- Initially, it was difficult to recruit patients with early untreated RA. I established connections with the rheumatology consultants Dr McKay and Dr Gray, who agreed that patients from their Early Arthritis Clinic could be recruited for this study.
- Unfortunately, the quantity of B cells isolated initially was insufficient to process the samples further with bTMP and sonication. Therefore, we adjusted the protocol (increase in the number of isolated B cells and glycogen marking of B cells) to proceed with molecular experiments.
- We confirmed recovery of bTMP-treated DNA from B and T cells but, due to time constraints, we could not finalise microarray analyses. Dr Naughton and I have set up a schedule to finalise these analyses.

(C) COLLABORATIONS ESTABLISHED

- We have been involved and established links with the clinical and research rheumatology team at the Western General Hospital with Dr McKay (who runs the early Rheumatoid Arthritis Clinic) and the research nurse (Barbara Robson).
- I have collaborated closely with Professor Nick Gilbert and Dr Catherine Naugton at the Institute of Genetics and Molecular Medicine.
- The Gastroenterology Team (Professor Satsangi) and in particular Dr Alex Adams assisted in cell isolation.

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

None to date.

(E) ACKNOWLEDGEMENTS

I thank the Lorna Smith Charitable Trust for providing me with a research grant.

Fellowship Report

Investigation into the effect of physical modalities on antibiotic efficacy in an *in vitro Staphylococcus aureus* biofilm model

Shao-Ting Jerry Tsang
Department of Orthopaedic Surgery, University of Edinburgh
Joint RCSEd/Cutner Research Fellowship in Orthopaedics
November 2016 to October 2017

LAY SUMMARY

Staphylococcus aureus is one of the leading causes of hospital-acquired infections. It is responsible for 60–70% of infections of surgical implants and prostheses in orthopaedic surgery, amounting to £120–200 million annually in total costs for hospital treatment. Its ability to develop further resistance to a diverse range of antimicrobial compounds threatens to halt routine elective implant surgery.

One strategy to overcome this problem is to look beyond traditional antimicrobial drug therapies and investigate other treatment modalities. Physical modalities, such as ultrasound, electromagnetic fields and near-infrared laser therapy, are poorly explored, but preliminary work has shown potential benefit, especially if combined with antibiotics.

By developing a novel therapy and enhancing the efficacy of antibiotics in the treatment of prosthetic joint infections there is great potential to reduce morbidity and treatment time. This research represents the initial phase of work to identify a novel therapy in the treatment of resistant *Staphylococcus* infection as well as optimal methods for enhancing antibiotic action in the context of prosthetic joint infections.

GRANT REPORT

(A) CLINICAL AND SCIENTIFIC SIGNIFICANCE OF ADVANCES MADE

The overall aims of our study were to:

(i) Describe the biochemical pathways involved in the bactericidal effect of "near-infrared" laser therapy on *S. aureus* within an *in vitro* biofilm model.

(ii) Investigate the synergism of other forms of intervention, such as sonication, when applied separately or in combination with near infrared laser therapy, on the antimicrobial susceptibility of *S. aureus* within an *in vitro* biofilm model.

(iii) Further describe the molecular mechanisms utilised in the development of antimicrobial resistance within *S. aureus* in a biofilm.

Initially, we focused on optimisation of the proprietary dissolvable bead biofilm assay, which was used to evaluate antibiotic efficacy in combination physical therapies. The optimised assay was used subsequently to evaluate: (i) putative anti-staphylococcal biofilm antibiotic combinations used in periprosthetic joint infection (PJI); (ii) the antibiofilm activity of acetic acid in PJI management.

We found that gentamicin and daptomycin were the only effective single-agent antibiotics, if used at clinically achievable and locally delivered concentrations, against *Staphylococcus* biofilms. Also, we were the first to report that addition of a bacteriostatic antibiotic (clindamycin, linezolid, or rifampicin), at commonly used concentrations, could antagonise the ability of gentamicin to eradicate *Staphylococcus* biofilms. The antagonistic effect of the bacteriostatic antibiotics tested in our study has clinical importance because they are commonly combined with gentamicin used in surgery for PJI.

Through a collaboration with Leonardo (Selex Medical), I was involved in several clinical pilot studies which sought to establish the efficacy of preoperative methicillin-resistant *Staphylococcus aureus* (MRSA) eradication therapy before joint replacement. These pilot studies were done in collaboration with Professor Tim Walsh (Department of Anaesthesia, Critical Care, and Pain Medicine, University of Edinburgh). We found that current MRSA decolonisation regimens were well-tolerated and

effective for *S. aureus* decolonisation for the anterior nares and groin. Also, the decolonisation effect was preserved for ≥10 days after completion of the regimen.

Investigation into the antibiotic potentiation effect of low-intensity ultrasound in the eradication of *S. aureus* biofilms was initiated. Early *in vitro* studies demonstrated an effect. Further work to characterise the mechanism of action and to evaluate the effects on mammalian tissue using a novel electrical impedance method is being conducted. The ultrasound configuration used in these *in vitro* studies is licenced for clinical use for management of fracture non-union and could be translated quickly into a clinical treatment.

(B) PROBLEMS ENCOUNTERED AND STEPS TAKEN TO OVERCOME THEM

The requirement for the laser therapy to be optimised further delayed characterisation of the biochemical pathways associated with the bactericidal effect of near-infrared laser therapy. Once the therapy has been optimised further it is hoped that this line of investigation can be revisited. This collaboration with industry has given me valuable insights into the processes involved in delivering a novel medical technology for clinical use.

(C) COLLABORATIONS ESTABLISHED

Inter-departmental collaborations within the University of Edinburgh were established with: Professor Maurice Gallagher (School of Biological Sciences); Professor Tim Walsh (Department of Anaesthesia, Critical Care, and Pain Medicine); Professor John Pleveris (Department of Gastroenterology and Hepatology); Dr Pierre Bagnaninchi (Centre for Regenerative Medicine).

Industrial collaborations were formed with Leonardo (Selex Medical, Edinburgh) and Ideal Medical Solutions (Tadworth, Surrey).

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

Publications

- Dall GD, Tsang STJ, Gwynne PJ, et al. The dissolvable bead: a novel *in vitro* biofilm model for evaluating antimicrobial resistance. *J Microbiol Methods* 2017;142:46–51.
- Tsang STJ, Ting J, Simpson AH, et al. Outcomes following debridement antibiotics and implant retention for periprosthetic hip infections: a review of cohort studies. *Bone Joint J* 2017;99:1458–1466.
- Tsang STJ, McHugh MP, Gwynne PJ, et al. Underestimation of *Staphylococcus aureus* (MRSA and MSSA) carriage associated with standard culturing techniques: one third of carriers missed. *Bone Joint Res* 2018;7:79–84.
- Dall GD, Tsang STJ, Gwynne PJ, et al. Unexpected synergistic and antagonistic antibiotic activity against *Staphylococcus* biofilms. *J Antimicrob Chemother* 2018; Mar 14.
- Tsang STJ, McHugh MP, Guerendiain D, et al. Evaluation of *Staphylococcus aureus* eradication therapy in orthopaedic surgery. *Med Microbiol* 2018;67:893–901.
- Tsang STJ, Gwynne PJ, Walsh TS, et al. Antibiofilm activity of acetic acid in the management of periprosthetic joint infection. *Bone Joint Res* [submitted November 2017].

Presentations

- Tsang STJ, McHugh MP, Guerendiain D, et al. Evaluation of *Staphylococcus aureus* eradication therapy in orthopaedic surgery. European Orthopaedic Research Society (EORS) congress, 2017. Oral presentation.
- Tsang STJ, McHugh MP, Guerendiain D, et al. Underestimation of *Staphylococcus aureus* carriage associated with standard culturing techniques. EORS congress, 2017. Poster presentation.
- Tsang STJ, McHugh MP, Guerendiain D, et al. Underestimation of *Staphylococcus aureus* carriage associated with standard culturing techniques. Scottish Committee on Orthopaedic and Trauma meeting, 2017. Oral presentation.
- Tsang STJ, McHugh MP, Guerendiain D, et al. Underestimation of *Staphylococcus aureus* carriage associated with standard culturing techniques. British Orthopaedic Research Society congress, 2017. Poster presentation.
- Ting J, Tsang STJ, Simpson AH, et al. Outcomes following debridement antibiotics and implant retention for periprosthetic hip infections: a systematic review and meta-analysis. British Hip Society meeting, 2017. Oral presentation.

Higher degree

Doctorate in Trauma and Orthopaedic Surgery, University of Edinburgh. Enrolled November 2016.

Fellowships
Clinical Research Fellowship, University of Edinburgh.
With Professors Hamish Simpson, Timothy Walsh,
and Maurice Gallagher
November 2017–October 2019

BORS/Bone & Joint Research International Travelling Research Fellowship for Young Investigators
North America, February/March 2018

(E) ACKNOWLEDGEMENTS
I acknowledge the support received from Leonardo (Selex Medical) and Ideal Medical Solutions.

Fellowship Report

MicroRNA biomarkers in antineutrophil cytoplasmic antibody vasculitis: a prospective cohort study

Tariq Farrah
Centre for Cardiovascular Science, University of Edinburgh
Lorna Smith Charitable Trust Fellowship
August 2016 to August 2017

LAY SUMMARY

Antineutrophil cytoplasmic antibody (ANCA) vasculitis involves inflammation of small blood vessels. This inflammation can be severe and, occasionally, life-threatening. The diagnosis of ANCA vasculitis is often delayed and, even with treatment, the condition can return.

I aimed to develop a blood test using microRNAs (miRs) to improve the diagnosis and treatment of ANCA vasculitis. I collected blood samples from ≈50 patients before and after treatment for newly diagnosed or relapsing vasculitis. Initial studies using these samples enabled identification of a potentially unique pattern of miRs that are low when vasculitis is active but which rise with successful treatment. Testing for these changes may be better than current blood tests and I am undertaking further experiments to explore this. The Lorna Smith Charitable Trust Fellowship has been fundamental in developing this work, and has provided a strong platform from which I can continue my research to improve the lives of patients with ANCA vasculitis.

GRANT REPORT

(A) CLINICAL AND SCIENTIFIC SIGNIFICANCE OF ADVANCES MADE

I undertook a prospective study of patients with ANCA vasculitis to develop circulating miRs as novel biomarkers of disease activity.

First, I generated a highly phenotyped set of pre- and post-treatment blood samples from patients with a new diagnosis or relapse of ANCA vasculitis. I took samples from ≈50 patients and sought to identify a unique miR panel of disease activity (derivation study). I carried out RNA sequencing on these paired samples to identify a panel of miRs that showed significant changes after successful treatment of ANCA vasculitis. This approach is unique and allows profiling of the global change of miRs in ANCA vasculitis. These data can be used in my prospective clinical study while facilitating back-translation into relevant animal models for mechanistic work.

Initial RNA sequencing identified a unique pattern of miR expression in remission compared with active disease.

I identified a panel of eight miRs which changed following resolution of active disease. This pattern of miR expression has not been described in vasculitis before and may represent a potential novel biomarker panel which I will explore in my ongoing prospective study (validation study).

I have started prospective recruitment of a separate cohort of patients with ANCA vasculitis at the time of the diagnosis or relapse. I am collecting samples of blood and urine from these patients every month from the time of diagnosis. This strategy will allow exploration of the relationship between my derived miR panel and disease activity over time. I have recruited patients presenting with acute kidney injury and sepsis to act as control groups. To date, I have recruited ≈50 patients and look forward to conducting interim analyses using RNA quantitative polymerase chain reaction (qPCR) for my miR signature panel. This will validate my derived miR panel in a prospective cohort.

I have also initiated a series of exploratory experiments to ascertain if miRs may have additional pathogenic effects as well as acting as biomarkers. miRs are post-transcriptional regulators of cell behaviour and exist in several forms in the circulation, including being packaged into extracellular vesicles (ECVs). I collaborated with another doctoral student in my laboratory to isolate ECVs from human plasma and explore if they are taken up by macrophages and renal tubular cells. Then,

we explored whether ECVs from patients with ANCA vasculitis can change their behaviour compared with ECVs from healthy controls. Our data showed that ECVs are taken up by macrophages and renal tubular cells. However, to date we have not identified differential effects between ECVs from patients with ANCA patients and healthy controls. Further experiments are planned to explore this relationship further.

My prospective study is part of a 2-year project with data-linkage for up to 5 years. My miR panel will be correlated with clinical outcomes such as hospital admission, renal failure and death. This linkage of miR to longer-term clinical outcomes is another unique aspect of this study, and was made possible through the award of the Lorna Smith Charitable Trust Fellowship.

(B) PROBLEMS ENCOUNTERED AND STEPS TAKEN TO OVERCOME THEM

Engagement of patients and public in research can be challenging. My study relied on timely collection of samples, adequate population size and robust follow-up data over a long period of time. I worked hard to ensure that sample collection and follow-up took place in the least intrusive manner. Therefore, I scheduled follow-up visits for the same days as hospital appointments and taking of blood samples. Patients commented that they found this approach very convenient and that it was a significant contributing factor to their agreement to take part and remain engaged with the project.

RNA sequencing generates vast amounts of data that can be challenging to analyse. To assist with this, we formed a unique partnership with the Beijing Genomics Institute (a world-leading RNA sequencing laboratory). They provided invaluable processing, data analysis and bio-informatics support to make sense of the unique miR data we generated.

(C) COLLABORATIONS ESTABLISHED

My research aims to establish miRs as novel circulating biomarkers in ANCA vasculitis. My studies began through a derivation study followed by a prospective validation study. The next stage was to validate my miR panel in an external cohort. Thus, I established collaboration with Dr Neil Basu, a leading academic rheumatologist who heads the Aberdeen Vasculitis Service. His research team has begun the collection and banking of samples using our shared standard operating procedures from ANCA-vasculitis patients in Aberdeen. This will provide an independent external cohort from which I can test my identified miR panel.

Our laboratory has several scientists interested in miRs, and we formed a strong partnership with the Beijing Genomics Institute. This partnership was vital to processing and analysing my miR samples and will continue to grow as my project develops.

In addition, I developed collaborative interests with scientists within my own laboratory who share an interest in miRs. We started some initial mechanistic studies to complement my clinical work and this will continue over the medium to long term.

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

The Lorna Smith Charitable Trust Fellowship has allowed me to begin doctoral studies exploring miRs as biomarkers in ANCA vasculitis. This funding has enabled me to generate novel pilot data for an application to the MRC for a longer-term Clinical Research Training Fellowship.

Publications

- Farrah TE, et al. Retinal optical coherence tomography and cardiovascular risk: old concepts, better tools, new horizons. *J Am Soc of Nephrol* [under revision].
- Farrah TE, et al. Endothelin receptor antagonism improves lipid profiles and lowers PCSK9 in patients with chronic kidney disease. *J Am Soc of Nephrol* [submitted].
- Farrah TE, et al. Plasma galectin-3 and risk of incident chronic kidney disease. Letter to *Kidney Int* [in press].
- Anand A, Farrah TE, et al. Serial troponin measurements to monitor cardiovascular risk and response to novel cardiovascular therapies. *Clin Chem* [submitted].

(E) ACKNOWLEDGEMENTS

I acknowledge the ongoing support and guidance of my supervisors, Dr Neeraj Dhaun and Dr James Dear. I also acknowledge my collaborators, Kathleen Scullion and Dr Neil Basu, who have helped tremendously over the past year and hopefully will continue to do so during our upcoming projects.

Fellowship Report

Development of a biodegradable membrane to be used with skin explants for a one-stage, full-thickness reconstruction of skin defects

Kavita Sharma
Department of Material Sciences and Engineering, University of Sheffield
Maurice Wohl Research Fellowship
August 2016 to August 2017

LAY SUMMARY

The aim of this project was to develop a synthetic biodegradable scaffold which mimics two of the key features of skin: rete ridges and a basement membrane. This was combined with small pieces of skin explants in the operating theatre for one-stage full-thickness skin reconstruction. The rete ridges provide physical protection for basal epithelial cells. The basement membrane is a three-dimensional (3D) structure of specialised collagens which act to tether basal keratinocytes to the underlying dermal collagen.

Tissue-engineered skin substitutes have not sought to replicate these features. Many 3D skin substitutes show poor epidermal–dermal adhesion (the epidermis can be lost if skin is subject to horizontal shear forces).

The current project combined US Food and Drug Administration (FDA)-approved materials (a combination of polylactic acid, polyglycolic acid and polyhydroxybutyrvalerate (PHBV)) to produce a biodegradable dermal substitute which breaks down within a few months. We hypothesise that this is more conducive to the regeneration of full-thickness skin.

GRANT REPORT

(A) CLINICAL AND SCIENTIFIC SIGNIFICANCE OF ADVANCES MADE

This project aimed to design and evaluate a novel biodegradable dermal alternative (composed of FDA-approved polylactic/glycolic acid and PHBV) to be combined with finely dissected pieces of split-thickness skin for use as a one-step approach for reconstruction of full-thickness skin defects.

As part of this project we wanted to: (i) evaluate how a novel method for spinning a basement-membrane substitute would encourage epithelial–stromal organisation; (ii) investigate if the inclusion of features approximating the dimensions of rete ridges would help in the outgrowth of epithelial cells into the scaffolds.

We were able to demonstrate first that the scaffolds allowed the proliferation and migration of keratinocytes and fibroblasts along their structure. Addition of a basement membrane 'mimic' retained its structure and function to define two separate layers to the skin (i.e., the epidermis and dermis). Addition of rete ridges, although mechanically strong, did not confer a 'migration' advantage to skin cells. As a result, we investigated: (i) use of fibrin and the optimal concentration of fibrin that would be appropriate to keep the explants on the scaffolds but facilitate skin-cell migration; (ii) the thickness of the dermis used in the biopsy and culturing of scaffolds with fibroblasts so as to simulate a more accurate model of the wound bed.

We found the optimal viscosity of fibrin arose from a certain concentration of fibrinogen and thrombin. At this concentration, the fibrin did not set too quickly nor was it too viscous to allow migration of skin cells through its structure. Use of fibrin was an essential aspect of this construct to keep the skin cells on the scaffolds for as long as possible. This prolonged contact had the aim of increasing the number of skin cells that leave the cut edge of the skin explants and migrate onto the scaffold.

Dermal thickness was found to be an important factor that influenced the outward migration of skin cells. Samples with more dermis resulted in the migration of fewer skin cells because the latter stayed within the niche containing fibroblasts. However, samples with very thin or little dermis had more outward migration of skin cells. As a result, we aimed to use skin biopsies with little or thin dermis in subsequent experiments.

Finally, we advanced our model by pre-seeding our scaffolds with fibroblasts. It is known that skin cells migrate from the cut edge of a skin explant if placed

on a wound bed due (at least in part) to growth factors and other stimulatory molecules secreted primarily by fibroblasts. This setup was not replicated *in vitro*. Therefore, by pre-seeding the scaffolds with fibroblasts, the model became a step closer to acting like a wound bed, and this indeed had a significant positive effect on the migration of skin cells from the explants onto the scaffold.

(B) PROBLEMS ENCOUNTERED AND STEPS TAKEN TO OVERCOME THEM

Despite templating the scaffold, there was no objective evidence that the skin cells had migrated significantly more than in the samples in which the templates (rete ridges) were not present. This observation led me 'back to the drawing board' to the model that we hoped to use in the hospital setting. This consisted of applying the templated trilayer scaffold onto a freshly debrided wound bed (full thickness), followed by minced skin explants (MSEs) from a small biopsy. The MSEs would be traditional pinch grafts and, when placed on wound beds, skin cells would migrate from the cut edges and eventually re-epithelise the wound. We were testing out this model *in vitro* so there were important differences that needed to be taken into account.

First, our *in vitro* model had some key differences to the proposed setup in clinical practice. The MSEs were not secured to the scaffold. In clinical practice, skin applied to a wound bed would be secured with sutures or glue. We then decided to investigate the use of fibrin as an agent to increase the contact time between the MSE cells and scaffolds. We also had to determine the optimal concentration of thrombin and fibrinogen (key components of fibrin) that would allow the minced skin to stay on the scaffold but facilitate the diffusion of skin cells through fibrin. Another key factor that facilitates the migration of skin cells from the cut edges of minced skin is signalling from the wound bed. Most wound beds consist of growth factors secreted by fibroblasts. Our translational idea was to have the scaffold on a wound bed so that these substances would act as stimulants for the migration of skin cells, and the scaffold would act as a facilitator. Therefore, to enhance our *in vitro* model, we pre-seeded the scaffolds with fibroblasts for 48 h before adding the MSEs. In this way, we attempted to create as close as possible an *in vivo* wound bed.

(C) COLLABORATIONS ESTABLISHED

None.

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

- British Burn Association meeting, April 2018.
- American Association of Plastic Surgeons meeting (October 2018).
- Submission of MD thesis in July 2018.

(E) ACKNOWLEDGEMENTS

- Maurice Wohl Research Foundation for their decision to support research in the development of tissue-engineered applications for burns and reconstructive surgery.
- The Royal College of Surgeons of Edinburgh, especially Mrs Cathy McCartney (Research and Grants Coordinator) for all her understanding and support throughout this research, without which I would not have been able to complete this work.
- Professor Sheila MacNeil for her guidance, support and commitment to the advancement of tissue-engineered skin in plastic, burns and reconstructive surgery.
- Dr Sabi Roman for his guidance and patience in helping me throughout the project, especially with respect to technical steps in which I lacked experience.
- Dr Naside Mangir for her guidance and support with respect to scientific concepts that I did not understand.
- Dr Atasham Raza for teaching me, over many days, immunohistochemistry and use of the confocal microscope.
- Mrs Victoria Giblin and Mr David Ralston (Consultant Plastic and Reconstructive Surgeons, Sheffield Teaching Hospitals) for clinical exposure and progression to allow a seamless re-transition back into clinical training.

Fellowship Report

Reliability of low-fidelity simulation models in acquisition of basic surgical skills: role of deliberate practice

Sotiris Papaspyros
Department of Cardiothoracic Surgery, Royal Infirmary of Edinburgh
FST/ASME Research Grant
August 2016 to October 2017

LAY SUMMARY

Surgical training has several limitations: a shorter working week for residents, increasing complexity of cases, emphasis on efficiency in the operating room, and mitigation of medical errors.

Acquisition of basic surgical skills can take place outside the operating room on low-fidelity, readily available simulation materials (e.g., bananas, potatoes, poached eggs).

Deliberate practice can provide the educational framework to achieve competence in surgical tasks (e.g., needle rotation, economy of movement, pace).

Over 9 months we recruited 30 junior doctors and medical students with minimal or no previous exposure to surgery.

We purchased an ironing board ('operating table'), needle holders, sutures and bananas. We explained to each participant the concept of 'deliberate practice'. We video-recorded and scored their attempts before and after 6 days of practice at home.

There was a significant improvement in all parameters measured: flow/rhythm, precision, rotation and time/pace. Twenty-eight participants improved their skills in all categories and could carry out the task more quickly with minimal hesitation, deviation, interruption or repetition.

This project provides compelling evidence that basic surgical skills can be taught reliably and learned using low-fidelity models and deliberate practice.

GRANT REPORT

(A) CLINICAL AND SCIENTIFIC SIGNIFICANCE OF ADVANCES MADE

This project has enhanced our knowledge and practice in surgical education and training from the perspectives of trainees and trainers.

Our work provides evidence on how low-fidelity simulation models can be used reliably to achieve significant progress through the early stages of the learning curve. Furthermore, it demonstrates the principles that must be observed for deliberate practice to be effective and efficient in the acquisition of surgical skills.

Accurate assessment of an individual's abilities at an early stage may be critical in his/her choice of career and whether he/she has a realistic chance of becoming an expert through deliberate practice.

The next step in this project is to identify the software that will support video-based motion analyses to quantify the parameters objectively. Our aim is to provide insight into how assessment methods can be optimised in terms of validity, reproducibility and cost efficiency.

(B) PROBLEMS ENCOUNTERED AND STEPS TAKEN TO OVERCOME THEM

We used video to record and score each participant. This was done on a visual scale and was open to observer bias for the three qualitative parameters measured. We could not identify the software required to establish an objective motion-analysis method.

(C) COLLABORATIONS ESTABLISHED

None.

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

- Presentation: Faculty of Surgical Trainers (FST) conference (Birmingham, 2017).

- Book chapter: Acquisition of Surgical Skills – from novice to master; a fresh perspective in *Recent Advances in Surgery*.
- Publication (Submitted): Diastolic learning: making the tacit, explicit. The role of low-fidelity simulation models and deliberate practice in the acquisition of basic surgical skills [submitted].

(E) ACKNOWLEDGEMENTS

This work was supported by the FST and ASME with a research grant.

Fellowship Report

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

Thomas Kurien
Academic Orthopaedics Trauma and Sports Medicine, University of Nottingham
MRC/RCSEd Clinical Research Training Fellowship
August 2014 to July 2017

LAY SUMMARY

Predicting which patients develop chronic postoperative pain and dissatisfaction after total knee arthroplasty (TKA) remains an elusive goal for orthopaedic surgeons worldwide. Pre-TKA osteoarthritis (OA) pain is not wholly due to joint disease but also central and peripheral sensitisation. Hence, improved understanding of a mechanism-based approach to pain in OA is required to improve patient outcomes after joint replacement.

Temporal summation of pain (TSP) is the perception of increasingly augmented pain evoked by repetitive noxious stimuli (≥0.33 Hz) and a measure of central sensitisation of pain. TSP has been shown to predict pain after TKA.

We conducted a prospective study on brain function using magnetic resonance imaging (MRI) with an age- and sex-matched healthy control group. We used a TSP paradigm with a novel cuff algometer in patients with knee OA awaiting TKA to identify the preoperative biomarkers of central sensitisation in the brain that predict poor outcome after TKA. We identified that the regions of the default mode network (DMN) and somatosensory regions of the brain are activated in patients who continue to report severe pain and central sensitisation 6 months after TKA.

We identified that the regions of the default mode network (DMN) and somatosensory regions of the brain are activated in patients who continue to report severe pain and central sensitisation 6 months after TKA.

GRANT REPORT

(A) CLINICAL AND SCIENTIFIC SIGNIFICANCE OF ADVANCES MADE

Increased neural brain activity in the secondary somatosensory area (S2) and impairment in DMN regions are imaging biomarkers in central sensitisation in knee OA. This neural activity is stimulated by chronic synovitis in the knee driving facilitation of the central integrative mechanisms of pain. Responders to TKA normalised their brain-related activity back in a similar pattern to healthy volunteers and showed no evidence of central sensitisation as assessed by quantitative sensory testing. Non-responders to TKA continued to report significant postoperative pain, lower Oxford Knee scores, continued facilitated TSP, increased S2 neural brain activity, impaired pain processing in the DMN along with postoperative bone-marrow lesions (BMLs) and synovitis contributing to post-TKA pain.

Our work is novel. We identified the 'neural signature' of TSP in knee OA and showed that brain-related changes to TSP are maintained in patients with chronic pain after TKA. Preoperative identification of OA patients with central sensitisation of pain and subsequent pharmacological treatment along with the eradication of painful BMLs and synovitis may reduce the number of patients developing pain after TKA significantly.

(B) PROBLEMS ENCOUNTERED AND STEPS TAKEN TO OVERCOME THEM

The first 6 months of my doctoral studies was spent developing an 'engineering box'. This box had to be MRI-compatible and generate knee compression in a patient to stimulate mild-to-moderate pain that could be used in the MRI scanner. After my Visiting Fellowship to the Centre for Sensory Motor Interaction (University of Aalborg, Denmark) and meeting with Professor Lars Arendt-Nielsen, we collaborated to use his validated cuff algometer, which is more accurate and observer-independent. This has been a huge success and has allowed me to generate some exciting data from my research.

(C) COLLABORATIONS ESTABLISHED

I established collaboration with Professor Lars Arendt-Nielsen (a world expert in pain medicine) in Denmark. I also undertook a Visiting Fellowship during this award at the Wellcome Trust for Mitochondrial Disease at the University of Newcastle under Professor Sir Douglass Turnbull, where some bone tissue from the knee was analysed. I hope that these collaborations will continue during my academic career.

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

- Doctorate – University of Nottingham July 2018.
- Further funding – BASK Knee Research Fellowship (£50,000) in 2014.
- Kurien T, Petersen KK, Arendt-Nielsen L, et al. Preoperative neuropathic pain like symptoms and central pain mechanisms in knee osteoarthritis predicts poor outcome 6 months after total knee replacement surgery. *J Pain* 2018; pii: S1526-5900(18)30291-8.
- Kurien T, Kerslake RW, Haywood B, et al. Resection and resolution of bone marrow lesions associated with improvement of pain after TKR. *Case Rep Orthop* 2016; 6043497.
- Kurien T, Reckziegel D, Cottam WJ, et al. Neural correlates of temporal summation in knee osteoarthritis pain: a preliminary fMRI study at 3T. *Osteoarthritis Cartilage* 2016; 24: S455.
- Kurien T, Price K, Pearson RG, et al. Manipulation and reduction of pediatric distal radial fractures in the emergency department: a 2.5-year study. *Bone Joint J* 2016; 98: 131–136.
- Salah O, Kurien T, Baker B, et al. Septic arthritis in the era of immunosuppressive treatments; old foe with a new friend? *Ann Royal Coll Surg* 2014; 96: e11–e12.

Prizes

- NIRA New Investigator Recognition Award Winner ORS 2018
- NIRA Winner, Orthopaedic Research Society, New Orleans, March 2018
- BORS/BJR International Travelling Fellowship for Young Investigators 2018
- International Research Fellowship to centres in the USA in 2018, awarded at BORS annual meeting, London 2017
- Andrew Sprowson Award for Translational Research (BORS)
- First Prize for a Podium Presentation at BORS, Imperial College, London, September 2017
- British Association for Surgery to the Knee
- First Prize for Best Podium Presentation at BASK, Southport, March 2017
- East Midlands Orthopaedic Research Prize
- First Prize for the Best Oral Presentation, Nottingham, December 2016
- Andrew Sprowson Award for Orthopaedic Translational Research
- Award and First Prize for Best Oral Presentation at the BORS conference, Glasgow, September 2016
- Orthopaedic Research UK (ORUK)
- First Prize at the British Orthopaedic Trainees Association conference, Best Oral Presentation Leicester June 2016
- Sue Watson First Prize in Medicine
- First Prize, Nottingham, May 2016
- BASK – President's Medal Prize
- First Prize and President's Medal for Best Oral Presentation at BASK, Liverpool, April 2016
- Royal Society of Medicine Second Prize
- Second Prize at Royal Society of Medicine conference, December 2015
- European Paediatric Orthopaedic Surgery Prize
- Poster Prize at European Paediatric Orthopaedic Society, Marseille, April 2015
- *Bone and Joint Journal* Best Paper Prize at BSCOS
- First Prize at British Children's Orthopaedic Society conference, Liverpool, March 2015
- Malkin Memorial Prize in Orthopaedics
- Academic Research First Prize in Orthopaedics, Nottingham, July 2014
- BASK/DePuy Research Prize in Orthopedics
- Awarded for Research into Pain in Osteoarthritis, 2014
- Nottingham MRI Prize
- University of Nottingham MRI Prize, September 2014

(E) ACKNOWLEDGEMENTS

- Professor Brigitte Scammell, University of Nottingham.
- Professor Dorothee Auer, University of Nottingham.
- Arthritis Research UK Pain Centre, Nottingham.
- The Royal College of Surgeons of Edinburgh.

Travelling Fellowship Report

Report for the Royal College of Surgeons of Edinburgh/Society of Oral and Maxillofacial Surgeons (SOMS) Head & Neck Oncology Fellowship

Laith Al-Qamachi
ST7 in Oral and Maxillofacial Surgery, Queen Elizabeth Hospital, Birmingham
30 November 2015 to 10 January 2016

Head and neck oncology and reconstruction have been an ever-growing interest since I was a Senior House Officer in Glasgow after my dentistry degree. This interest has been heightened during my higher training in the West Midlands, particularly in my last 2 years at the Queen Elizabeth Hospital in Birmingham. I was supported strongly by the team to facilitate this Travelling Fellowship. The latter requires a semi-independent operator with good decision-making skills to facilitate comprehensive, hands-on training.

My Travelling Fellowship was at the Ninth People's Hospital, Jiaotong University, Shanghai, China. My supervisor was Professor Chen Ping Zhang. The Ninth People's Hospital is affiliated with the Dental and Medical School of Jiaotong University. It is a third-tier hospital with 1000 beds and provides tertiary care for all parts of China. The presence of other surgical specialties, such as Neurosurgery, provides good support to the Head and Neck team and a multidisciplinary approach to complex cases such as skull-base tumours.

One hundred and eighty beds are allocated to the Maxillofacial team. Seventy-five of these beds across two floors along with 10 intensive treatment unit (ITU) beds and 12 high-dependency unit (HDU) beds are allocated to the Oncology Group. The Maxillofacial Team is divided into three groups: Head and Neck Oncology; Cleft and Craniofacial; Temporomandibular Joint (TMJ) and Trauma. Each group has its own trainees and on-call system. The trainee sub-specialises as soon as he/she graduates from the School of Dentistry. This is a fundamental difference to the structure of a Maxillofacial team in the UK and in Europe. The Head and Neck Oncology and Plastic Surgery teams are the most influential within the hospital based on clinical research activities and financial input.

The Head and Neck Oncology Group is divided into nine teams. In general, each team comprises a MSc student, junior registrar, senior registrar, an attending surgeon (similar to a junior consultant) and a fellow, and is led by a professor. Most of the registrars have an MD or a doctorate and several publications. This reflects the policy of the department and hospital of encouraging clinical research and publication. The hospital awards a good doctoral thesis with an overseas research grant or Clinical Fellowship to internationally renowned centres such as MD Anderson in the USA. The department has educational and research agreements with some units worldwide. Recently, it was approached by the University of Moscow to facilitate microvascular training courses to Maxillofacial and Plastic Surgery trainees from Russia.

Approval of the unit as a training centre by the Royal College of Surgeons of Edinburgh has been one of its most prestigious achievements according to Professor Zhang (Head of Department). The department gets fellows from around the world, mainly via the AO Foundation and the International Association of Oral and Maxillofacial Surgeons.

Most of the cases were complex salvage surgery for patients treated primarily elsewhere in China. Some referrals were for secondary reconstruction. Dealing with complex salvage resections and reconstructions added to my experience.

The nine teams undertake major surgical procedures 4.5 days per week with Thursday afternoon reserved for a multidisciplinary team (MDT) meeting to discuss new complex referrals. I was asked regularly to discuss a treatment plan for some of these patients. Typically, there were 2–4 free flaps a day. The department carries out ≈700 free flaps per year with a success rate of ≈98.5%. This is an impressive record considering that most of these cases are salvage surgery and/or second recurrence.

However, most patients do not go through an MDT process. The individual surgeon makes all the decisions and resections are very radical. Disease stage and prognosis are taken into account before reconstruction. Hence, many mandibles would not be reconstructed primarily. This is related (at least in part) to the patient's decision from a financial viewpoint (patients must pay for most of the treatment cost). It is crucial to reflect on this latter point in comparison with the UK NHS, which pays for all treatment (including long-term and expensive rehabilitation). It made me so proud to be trained to work as a Head and Neck Surgeon within the NHS and, hopefully, provide our patients with advanced and comprehensive treatment.

On my first day at the hospital a team of resident doctors who speak good English was waiting for me to arrange my licence to practise and other hospital paperwork. On my second day, I had a meeting with Professor Zhang to set up my educational programme and the expected outcomes for the 6 weeks. My day started at 07.30 and ended when the operating team finished. I think this commitment was noted by the teams and facilitated my progress from an assisting surgeon to an operating surgeon.

I was assigned to three teams who operated 4 days a week. Occasionally, if a team had no major reconstruction on a particular day, I was moved to another team to fulfil my 'educational requirement'!

Initially, I was observing/assisting in the operating theatre for a few days. This was combined with comprehensive daily case-based discussions at the morning rounds. The latter was crucial in enabling me to participate actively in various aspects of the surgical procedure. I requested to be on the reconstruction (rather than the resection) part of the team. I was allowed to harvest a few flaps and undertake microvascular anastomosis under supervision (Figure 1). Their main 'workhorse' flaps are similar to those of most OMFS units in the UK, including the anterolateral thigh, radial forearm and fibula free flaps, along with pectoralis major flaps.

Considering the complexity of cases, operating with various surgeons and the language barrier, this was a huge learning curve and a wonderful experience. I created a logbook to register and reflect on these procedures. This was approved by Professor Zhang at the end of my Travelling Fellowship.

My learning experience did not stop at the operating room. I also had opportunities to improve my management, teaching and research skills. Upon arrival, I noted two patients who had suffered deep-vein thrombosis after major resection and reconstruction. The unit had no guidelines in terms of venous thromboembolism (VTE) prophylaxis. Even simple measures such as early mobilisation, hydration and graduated compression stockings were not applied. This strategy is based on studies showing that people from Asia-Pacific are genetically less prone to VTE. However, the department had noted an increasing problem of VTE over the previous 2–3 years. I was involved actively in auditing VTE prevalence in 2000 patients who underwent free flap reconstruction. Then, I organised a meeting with nursing staff, orthopaedics surgeons and vascular surgeons (who manage established VTE in the hospital). The meeting was led by Professor Xu. I delivered a presentation and proposed an initial thromboprophylaxis strategy. The department has implemented my strategy into clinical practice. We have drafted a manuscript which will be submitted to a relevant journal shortly.

I delivered a presentation entitled 'OMFS Training in the UK' that highlighted the main differences in the training pathway between the UK and China. A trainee in China must declare a sub-specialty interest in OMFS (oncology, deformity or TMJ/trauma) as soon as he/she leaves dental school and joins the OMFS team. Hence, they are not trained in all aspects of OMFS like UK candidates. The other part of the presentation explained the role of the Royal College of Surgeons of Edinburgh in setting standards for surgical training nationally and internationally.

I was invited by Professor Ji Tong to set procedural assessment forms similar to the Intercollegiate Surgical Curriculum Programme in the UK. This was to help the department in setting a standardised method of training and assessment.

Overall, this Travelling Fellowship was a highly productive, hands-on experience with a heavy caseload. However, the trainee must be towards the end of his/her training and have sufficient experience as a semi-independent surgeon.

I would like to express my gratitude to the Royal College of Surgeons of Edinburgh and Scottish SOMS for awarding me this Travelling Fellowship. I also thank the Director of the Department, Professor Zhang, for his support and teaching throughout my time in Shanghai. His generosity was never-ending. Special thanks are extended to Professors Xu and Ji for their training and support. Also, I would like to thank my department at the Queen Elizabeth Hospital for facilitating my study leave. Finally, huge thanks are extended to my daughter and wife

for their massive support while I spent Christmas and New Year away from them.

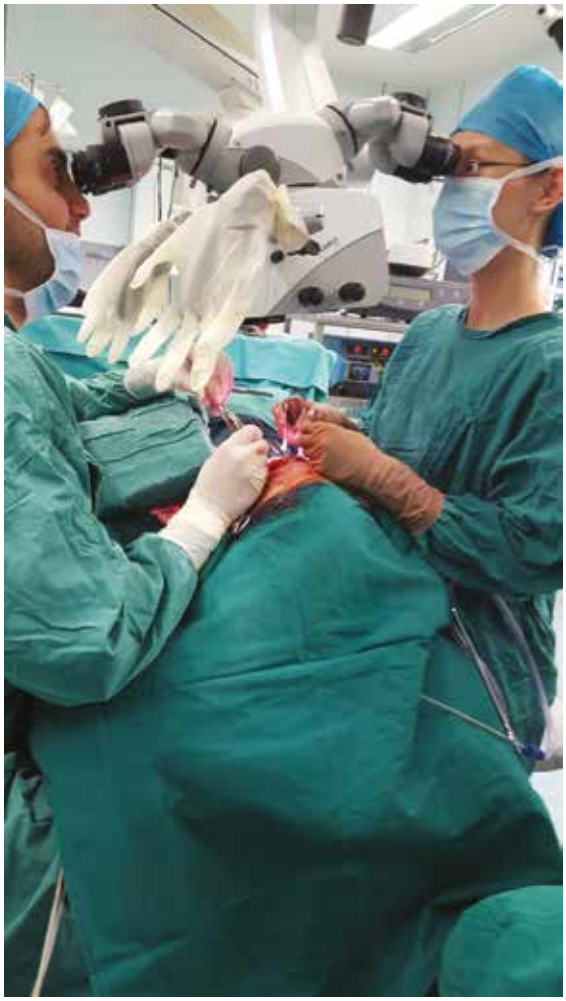


Figure 1: Harvesting flaps and undertaking microvascular anastomosis under supervision.

Travelling Fellowship Report

Report for the Cutner Travelling Fellowship in Orthopaedics 2016

Peter Domos
Specialty Registrar Trauma and Orthopaedics ST8, Shoulder Fellow, Peterborough City Hospital, Cambridgeshire
January to June 2016

Being awarded the Cutner Travelling Fellowship in Orthopaedics by the Royal College of Surgeons of Edinburgh gave me the amazing opportunity to travel within Europe and spend time with leading shoulder surgeons.

Between January and June 2016 I was at the Clinique Orthopedique Santy, an internationally renowned orthopaedic and sports injury clinic (Fédération Internationale de Football Association (FIFA) medical centre of excellence) in Lyon, France (Figure 1). It was founded in January 2006 by Dr Gilles Walch and Dr Pierre Chambat, and is dedicated to sports injuries and orthopaedic surgery. There are five shoulder surgeons (three specialising in lower-limb sports injuries and two in lower-limb arthroplasty) and three spinal surgeons within this group.

All members use operating theatres in the nearby Hôpital Privé Jean Mermoz (Figure 2). This private hospital has more than 150 surgical beds and 24 operating theatres, almost half of which are dedicated to trauma and orthopaedic surgery. It was ranked as the second-best private hospital in France, and 1500 shoulder procedures are undertaken there every year.

I was hosted by Drs Gilles Walch (Figure 3A), Arnaud Godeneche (Figure 3B) and Lionel Neyton (Figure 3C). All are internationally acclaimed shoulder surgeons and they and their team are at the forefront of recent developments in shoulder surgery because they have introduced major innovations and pioneering concepts. Their work has led to several advancements, and they use the latest evidence-based surgical treatments to improve patient care.

Figure 1: Clinique Orthopedique Santy, Lyon, France.

Figure 2: Hôpital Privé Jean Mermoz.

A B C

Figure 3: In the operating room with Drs Walch (A), Godeneche (B) and Neyton (C).

My weekly timetable comprised 1.5–2 days of outpatient clinics and 2–2.5 days of operating time. The clinic day usually commenced at 08.30, and at least 12–15 patients were reviewed per half-day session. Interestingly, routine follow-up cases were reviewed by a specialist sports medicine doctor and physiotherapist, so only major follow-up cases and new patients were booked for this clinic. The operating-theatre list began at 07.00. Two parallel operating rooms ran simultaneously, so 9–10 cases per day could be completed. There was a mixture of major shoulder arthroplasties and common arthroscopic methods. I scrubbed in for all cases as a first or second assistant (Figure 4A, B). I gained valuable exposure to primary and revision shoulder arthroplasties (RSAs; 60 cases), as well as to open anterior (Latarjet–Bristow), revision and posterior stabilisation (40 cases) and other arthroscopic methods (120 cases).

A B

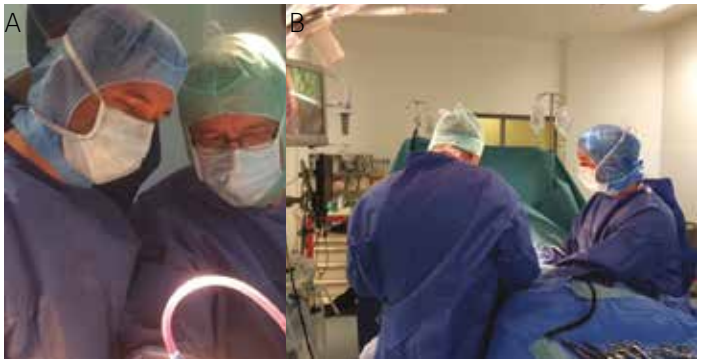


Figure 4: Drs Walch and Neyton (A) undertaking a total shoulder arthroplasty (B).

We had in-depth discussions regarding their approach to primary and complex degenerative, post-traumatic and inflammatory shoulder arthritis and their treatments and complications. I gained a good understanding of Dr Walch's new classification system of the glenoid deformity. I marvelled at his innovative three-dimensional computed tomography-based computer-navigated preoperative planning system for anatomic and reverse-shoulder arthroplasty, which aims at improving outcomes and reducing the risk of complications. I was also fortunate to witness use of patient-specific instrumentation (PSI) for complex preoperative glenoid bone loss/deformity (Figure 5).

I gained valuable insights into how they coordinate, collate and analyse data for multicentre projects. I was involved in several critical appraisals for pre-published articles and carried out several research projects (Latarjet–Bristow procedure for middle-aged and adolescent patients). I was also part of a research project involving seven shoulder-specific centres to investigate the mid-term results of RSAs in several different aspects. This provided me with some international presentations, as well as book chapters at Nice Shoulder Course 2016.

The mid-term results (minimum 5-year follow-up) of RSA for acute fractures were investigated out of 1953 procedures. A total of 131 cases were identified (7%) but only 39 patients were available with a mean age at follow-up of 77 months and 83 months. Ten percent of patients had complications (instability, humeral fracture) and 7% had to undergo a reoperation. The mean Constant Score was 60 points and 98 points when adjusted for age and sex, respectively. The mean Subjective Shoulder Value was 79%. The mean active elevation and external rotation (ER) were 128° and 12°, respectively. The prevalence of tuberosity union was 77% and glenoid notching was diagnosed in 51% of patients. An anterosuperior approach using a fracture-specific humeral stem improved healing of the greater tuberosity significantly, which led to better functional outcomes (Constant Scores and ER), but notching and age had no influence.

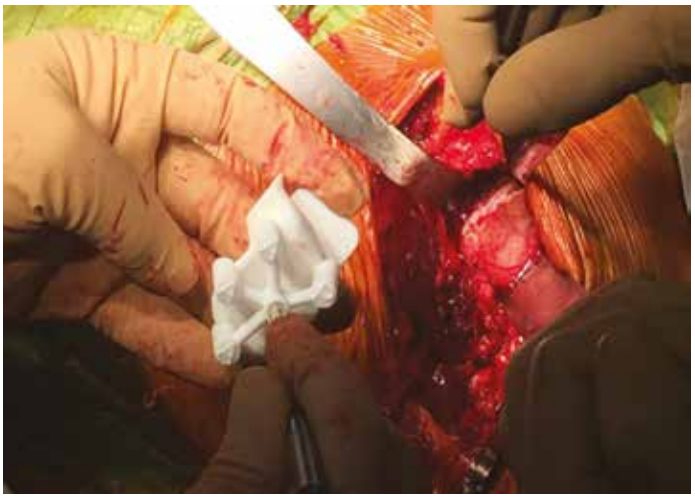


Figure 5: PSI for glenoid deformity.

I was welcomed into the International Shoulder Fellow Group (Figure 6). I set up and organised weekly journal club evening meetings to review the latest landmark studies on shoulder surgery, which were very informative.

Figure 6: In the operating theatre with Drs Walch and Neyton as well as other members of the International Shoulder Fellow Group.

We were also invited to attend monthly meetings on shoulder surgery in which all shoulder surgeons from Lyon and surrounding regions (even as far as Switzerland) could discuss complex cases and their management.

I would like to thank my hosts and the Royal College of Surgeons of Edinburgh for this unique and truly educational experience.

Travelling Fellowship Report

Report for the Cutner Travelling Fellowship 2017

Paul Monk
ST8 in Orthopaedics/NIHR ACL, Royal Berkshire Hospital, Reading

This report summarises my experience on the Cutner Travelling Fellowship to UniSports and the Auckland Bioengineering Institute at the University of Auckland in New Zealand.

UniSports is a high-volume academic orthopaedic unit regarded as a centre of excellence for its academic and clinical work. UniSports is a FIFA-accredited unit. It has one of the largest and most comprehensive databases for orthopaedic treatment for sports injuries in the world. UniSports undertakes basic science, orthopaedic engineering and clinical research relating to the prevention, diagnosis and management of early arthritis.

During this Cutner Travelling Fellowship, I undertook clinical and academic roles. At the clinical level, my mentors were Bruce Twaddle, Mike Rosenfeldt, and Stewart Walsh, under whose tutelage I have become proficient in the management of sport-based orthopaedic conditions, especially those of the shoulder and knee.

My research focused on improving the outcome of high tibial osteotomy. In collaboration with the Department of Orthopaedic Engineering we developed a novel method to calculate an improved, safe, three-dimensional correction of the weightbearing line. High tibial osteotomy has proven efficacy and good mid-term results for up to 10 years. Many surgeons aim to correct the position of the weightbearing line to a specific point (e.g., Fugisawa) but the surgical delivery of the preoperative plan remains unreliable. The effect of under- and over-correction on intra-articular meniscal and cartilage stress remains undefined. Hence, the aim of our study was to use finite element models to determine the effect of altering the position of the mechanical axis on compartment stresses in 'virtual' osteotomies.

Subject-specific finite element models were developed by combining geometries from 7-T MRI scans with boundary conditions representing joint loads from ground reaction forces during heel strike of level walking (Figure 1). Baseline stresses and pressures of menisci, tibial and femoral cartilage were calculated using patient data from kinetic and kinematic gait analyses. Then, progressive osteotomies were simulated *in silico* to shift the weightbearing line from the native alignment in 2° increments in coronal and sagittal planes. Changes in calculated stresses and pressures were recorded, and calculation of the optimal correction to neutralise the forces in the compartments was done.

Mean index loading profiles from gait data were 729 N with a varus of 32.3 Nm to a valgus of 10.56 Nm. Consistently higher stress distributions (10%) were illustrated by the menisci than the cartilage. Opening wedges of 2° increments decreased stresses in the medial compartment by 24%, whereas an under-correction of 2° caused a mean increase in medial stress of 41%. Increasing the posterior sagittal slope by 2° from neutral was associated with decreased medial compartment stress of 15% in menisci and cartilage. Increasing the change in the anterior sagittal slope by 2° was associated with a stress increase in the lateral compartment of 27%. The correction point of the neutralising weightbearing line was patient-specific.

These studies suggested that the correction zone within which a medial opening-wedge high tibial osteotomy can be done is patient-specific. The associated stress change with correction is highly sensitive with a mean stress change of 30% noted with 2° changes to the osteotomy plane.

Figure 1: Subject-specific models simulated incrementally can shift the mechanical axis in coronal and sagittal planes.

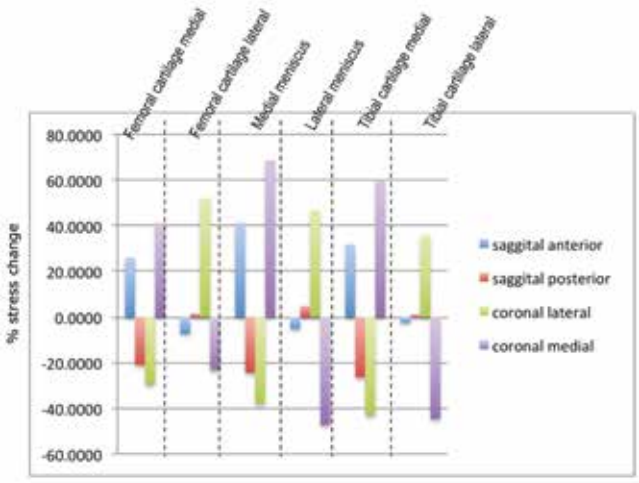


Figure 2: Stress changes at different compartments during tilting.

Use of these models has considerable potential for future planning of surgical procedures. It is likely that these models could be used for the planning of patient-specific osteotomies. It is hoped that by improving the accuracy of delivery of the preoperative plan we can improve the outcome of the procedure. Further work within our group is required to investigate the relationship between the improved surgical method and clinical outcome.

The Cutner Travelling Fellowship has been a wonderful opportunity to work in a new, dynamic environment. It has allowed a new collaboration with a world-class research facility and has tested my surgical skills. I would like to express my sincere thanks to the Cutner Travelling Fellowship for providing the support required to make this possible.

Travelling Fellowship Report

Report for the Sir John Steyn Travelling Fellowship in Urology

Aidan Noon
NIHR Clinical Lecturer in Urology, Royal Hallamshire Hospital, Sheffield
July 2013 to July 2015

I am very grateful to have received the John Steyn Travelling Fellowship in Urology. It allowed me to work at the University of Toronto for 2 years. The Fellowship was in Uro-oncology and was part of the Society of Uro-oncology Programme. This programme offers accreditation to several sites across North America and, at the time of my Fellowship, there were two centres in Canada which offered placements. The programme aims to equip the Fellow with enhanced academic understanding of all aspects of uro-oncology and to provide first-hand experience in dealing with various complex uro-oncological problems.

The first year of the Fellowship was spent undertaking research. Usually, there are three Fellows per year and each is assigned to a different consultant. I made contact with the Fellowship Director before starting my Fellowship because I was keen to undertake some research that centred on bladder cancer. I was very fortunate to be placed in the research team of Jeffrey Wrana at the Joseph & Wolf Lebovic Complex at Mount Sinai Hospital in Toronto.

I contributed to work on transcriptional analyses of bladder cancer. It was a fantastic opportunity to experience first-hand the complexities of 'personalised medicine'. I analysed tumour samples in the hope of identifying a 'molecular signature' that might help predict treatment response and patient outcome. I was also able to evaluate potential non-coding RNA species and their potential role as bladder-cancer biomarkers. I was able to work alongside some fantastic scientists and being part of this institution gave me a glimpse of what is possible in cancer research.

As well as the research I undertook in the first year I spent time in the Outpatients Department at Toronto General Hospital and Princess Margaret Hospital. The latter is a dedicated cancer centre and almost exclusively the patients seen have uro-oncological problems. This is obviously in contrast to the practice in many NHS institutions where, despite the sub-specialist interests of consultants, there is a mixture of general urological problems. Of particular interest was the Testicular Cancer Clinic, which was run jointly with members of the Oncology Team. This offered me new insights into how to manage this disease in a model, which I had not experienced previously in my training in the NHS. I was also able to spend time in a specialist Bladder Cancer Assessment Clinic, where a surgeon and clinical oncologist assessed patients and planned treatment from a flexible cystoscopy suite. Another standout feature of the programme was the weekly round, which was themed around interesting urological cases or dedicated to a specific cancer type (particularly renal cancer). This was excellent for highlighting unusual cases and, unlike in MDT meetings in the NHS, in which a high volume of patients is discussed, a small number of detailed cases had high educational value.

My second year was spent furthering my surgical training. For 4 out of 5 days I did complex oncological surgical procedures with the remaining day in clinic or doing research. One 4-month block was spent in a different part of Toronto at Sunnybrook Hospital, where I was attached to the Uro-oncology team. Surgery offered a variety of experience, and for 1 day per week I did robotic surgery. The other days were given to open prostatectomy, cystectomy and resection of renal tumours. Approximately once a fortnight there was dissection of retroperitoneal lymph nodes, which I had not been exposed to before my Fellowship. The consultants for whom I worked also offered intraoperative consultations to other members of the surgical team and often worked jointly, particularly in post-radiation exenterative surgery (e.g., sarcoma surgery). It was

a fantastic experience to be involved in these more complex cases.

This Fellowship was very demanding but offered a huge boost to my learning and confidence. It has set me up for my current position as a pelvic oncologist at Sheffield Teaching Hospitals NHS Foundation Trust. I extend my thanks to everybody associated with the John Steyn Travelling Fellowship in Urology, which was of huge benefit to myself and the patients under my care.

Travelling Fellowship Report

Report for the John Steyn Travelling Fellowship in Urology

Alastair Lamb
Clinical Lecturer/Hon SpR, Addenbrooke's Hospital, Cambridge
2016 to 2017

The Peter MacCallum Cancer Centre (PMCC) is part of the Victorian Comprehensive Cancer Centre in central Melbourne. PMCC is the only comprehensive cancer centre in the southern hemisphere and, until 2011, was the only public hospital in Australia to offer robotic surgery.

PMCC offers a full range of surgical, radiotherapy and chemotherapy modalities and treats 31,000 public and private patients every year, including 10,000 new patients. The Genitourinary Oncology Team spans each of these modalities and includes a four-consultant Urology Unit within the Division of Cancer Surgery: Mr Jeremy Goad, Professor Nathan Lawrentschuk, Mr Daniel Moon and Professor Declan Murphy. Each year, this team undertakes ≈110 robot-assisted radical prostatectomies (RARPs), 40 robot-assisted partial nephrectomies (RAPNs), 25 assorted pelvic exenterations as part of a multi-specialty team, 120 transperineal biopsies, and an assortment of retroperitoneal lymph-node dissections and partial bladder/prostate procedures alongside other diagnostic cancer work.

Although this was a Robotic Surgery Fellowship and based predominantly at PMCC, there was plenty of opportunity to experience the wider scope of urology across Melbourne. This included the full range of teaching-hospital trauma and emergency urology during monthly on-call sessions at the Royal Melbourne Hospital (the main teaching hospital at the University of Melbourne with ≈600 beds and linked to PMCC by a footbridge). There were occasional visits to other teaching hospitals, such as Austin Hospital in Heidelberg (north-eastern suburb) and Monash Hospital in Clayton (south-eastern suburb) for interesting major cases or collaborative research visits. I also spent some time assisting (with some console time) the PMCC consultants as well as Professor Anthony Costello in private-sector hospitals, including Epworth Hospital (a large, fully comprehensive 531-bed hospital in Richmond, east–central Melbourne) and Cabrini Hospital (a smaller Catholic hospital in Malvern).

There is an interesting split between private and public healthcare in Melbourne. About 75% of RARPs are done in the private sector in this city. Most consultants spend over half their time in private-sector sessions with visiting medical officer privileges at one or more public hospitals. The public sector is funded nationwide by the Medicare system but state governments take responsibility for provision at a local level. The Medicare budget annually in Australia is $150 billion. The most recent NHS budget (2015/2016) was £120 billion. The population of Australia is 23 million versus 64 million in the UK. Given a generous exchange rate of 2 Australian dollars to 1 pound sterling and comparative coverage for the NHS of 95% versus 50% for Medicare, then Australians spend three times as much per capita on public healthcare compared with the UK. Either the Australians care a lot more about their health than the British, or we get very good value for money in the UK (if we accept a comparable service with equivalent outcomes).

The primary purpose of this Fellowship was to gain surgical skills (in robotic surgery in particular). I already had 70 h on the robot console and had undertaken nine complete RARPs before going to Melbourne. However, I was put through the 'Melbourne deconstruction protocol' with my technique being developed through focused modular training, videos and with a further 100 h on the robot, including 19 complete robotic cases (Figure 1). This included five RAPNs, a procedure I had never undertaken before arriving in Melbourne. With regard to RARPs, it was a privilege to learn from four of the best robotic surgeons in the world and to have my technique refined and challenged. While I was there, these surgeons continued

to change their techniques, modifying several procedures in light of published evidence.

Procedure	#
RARP (part)	67
RARP (complete)	14
RARP (assisted)	127
RAPN (part)	11
RAPN (complete)	5
RAPN (assisted)	31
Robotic Nephr.	1
Lap Radical Nephr.	1
Open Partial Nephr.	4
Cystectomies	6
Ileal Conduit	11
Radical Orchidect.	9
RPLND	2
AUS (male)	5
TURP	13
TURBT	21
Stents	66
Transperineal Bx	68
TOTAL (part/complete)	**296**

Figure 1: The number of procedures I carried out at PMCC.

RARP = Robot-assisted radical prostatectomy
RAPN = Robot-assisted partial nephrectomy
RPLND = Retroperitoneal lymph node dissection
AUS = Artificial urinary sphincter
TURP = Transurethral resection of the prostate
TURBT = Transurethral resection of bladder tumour

The index procedures in this Unit are RARP and RAPN, and it was pleasing to have completed all or part of 79 of these robotic procedures. However, it was the combined open cases that also make PMCC a fantastic place to train. The 25 open procedures (mainly pelvic but some upper tract) were an unexpected bonus. One of the prostatectomists, Daniel Moon, is also proficient in artificial urinary sphincter (AUS) placement. Usually, this procedure is carried out by reconstructive surgeons, but should probably form part of our skill-set given the incidence of iatrogenic incontinence after RARP. This was, therefore, another unexpected skill to learn during the year, although I am not yet independently proficient in this. Alongside these major cases, I took responsibility for several non-specialist operating lists. Hence, I notched up several transurethral procedures (TURPs) as well as providing a stent service for other Oncology teams in the hospital with patients who, for example, had obstructed kidneys due to locally advanced colorectal or gynaecological malignancies or metastatic breast cancer.

This was also a productive year for research. I continued to work on manuscripts from Cambridge University in clinical prostate cancer and basic science. I also helped to submit abstracts to the European Association of Urology, American Urological Association and British Association of Urological Surgeons as well as putting together nine abstracts for the annual PMCC Research Day. We published a systematic review on salvage robotic prostatectomy, two reviews on prostate-specific membrane antigen scanning, three opinion pieces, and have two further manuscripts under consideration/submission. One of the latter presents urological complications after TPE, a report on two decades of pelvic exenteration from PMCC (possibly the largest of its kind in the world). The other opinion piece addresses the trends in RARP management in Australia over the past decade: a state-of-the-art for robotic prostatectomy. Hence, 13 articles for the year have been published, three are under consideration and two are awaiting submission.

In addition, I had the opportunity to forge links with some important scientific-research partners based at Monash University and PMCC. They will be important collaborators in the work I hope to undertake as a clinician scientist in the future.

Although the Fellowship was a salaried post, the wages were insufficient to cover the cost of moving my family and living in Melbourne for 1 year. I am, therefore, tremendously grateful to The Urology Foundation and particularly to Mr Dennis Cope, whose generosity through the inaugural John Fitzpatrick Travel Scholarship made this Fellowship possible. I also received a small Fellowship Award from the Royal College of Surgeons of Edinburgh, courtesy of The John Steyn family, for which I am similarly grateful.

Travelling Fellowship Report

Report for the Sir James Fraser Travelling Fellowship in General Surgery 2016

Mohan Singh
ST8 Oesophagogastric Surgery, West Midlands
March to June 2017

In March 2017, I had the opportunity to go on a sabbatical to Queen Mary Hospital (QMH) and United Christian Hospital (UCH) in Hong Kong (HK). Over 35 days I observed Professor Simon Law at QMH and Mr David Leung at UCH. Being attached to two hospitals gave me the opportunity to observe four full-day resection lists each week, alongside endoscopy.

HK has a high incidence of oesophagogastric (OG) cancers, and Professor Law's OG Unit has exemplary long-term survival data and one of the lowest rates of morbidity and mortality after oesophagectomy in the world.

In this summary, I aim to compare what I have observed and learnt about surgical training and OG resections in HK with my experience in the UK.

Surgical training in Hong Kong

The structure of surgical training in HK is 1-year internship (Foundation Year (FY)1 level in the UK) then 2 years of basic surgical training (BST; core surgical training (CT)1-CT2), followed by 4 years of higher surgical training (HST; surgical training (ST)3–ST6), culminating in 1–2 years in a post-Fellow of the Royal College of Surgeons (FRCS) award. Surgical training in HK does not conform to the European Working Time Directive (EWTD) and demands a degree of resilience familiar only to pre-EWTD, Calman-trained surgeons in the UK. Interns, BSTs, HSTs and Fellows work 2.5 weekends a month, and when on-call they work continuously for 24 h (or 48-h weekends) and straight into a normal working day the next day.

FY1s in HK earn an equivalent of £6,000 a month, but this is offset by the high cost of living. FY1s–CT2s present at weekly morbidity and mortality meetings conducted in English with a prompt 07.30 start and attended by everyone, from professors to interns from most surgical departments (with the exception of Orthopaedics). In the OG Surgical Unit, there is a minimum of one oesophago-gastro-duodenoscopy (OGD) list every day, including weekends. Due to the large number of endoscopies, the entire team is involved, such that an FY2-equivalent in QMH will do >100 diagnostic OGDs on his/her own, albeit supported by Fellows and consultants running parallel sessions next door.

BSTs are taught to undertake bedside ultrasound so that they can scan for gallstones and free fluid when on-call, whereas HSTs are taught to carry out therapeutic OGDs and colonoscopies. Approximately 700 OGDs are done per month by Professor Law's unit, so all OG consultant surgeons in HK do endoscopic mucosal resection, endoscopic submucosal dissection, endoscopic ultrasound (EUS), radiologically inserted gastrostomy, manometry, stenting, dilatation and peroral endoscopic myotomy themselves.

Preoperative investigations for OG cancer

Instead of cardiopulmonary exercise testing, potentially operable patients with oesophageal/cardial cancer are sent for cardiology and anaesthetic assessments together with lung function tests, regardless of the intended operative approach. Although preoperative staging is done using CT, positron emission tomography, OGD and EUS, staging laparoscopy is done only on the day of resection.

Minimally invasive oesophagectomies (MIOs)

Professor Law is involved in all the oesophagectomies done at QMH. In this Unit, there are three OG Surgical Consultants, three post-FRCS Fellows, one HST and one BST. They undertake ≈50 oesophagectomies per year (80% of these as MIOs) and 75 gastrectomies per year (80% laparoscopically). The tumours in HK

are mainly squamous cell carcinomas (>90%) and mid-oesophageal, so a three-stage resection is done routinely with a cervical anastomosis. The full length of the oesophagus is mobilised. QMH does not practise centralisation for OG resections, but this is advocated by Professor Law.

The operating theatre has laminar flow and multiple pull-down screens (from the ceiling) complete with high-definition stack systems. All OG resections commence with an OGD and injection of a fluorescent dye (indocyanine green (ICG)) peri-tumourally (submucosally, Figure 1) for brightly fluorescent visualisation of regional lymph nodes (LNs) during thoracoscopic resection to ensure the completeness of lymphadenectomy (Figure 1b, c). Alternating between standard xenon white light and fluorescence mode during surgery is effortless by pressing a button on the endoscope for stunning contrast images which readily detect potential LNs lurking within the operative field. Professor Law is studying the correlation between ICG-positive (*in vivo* fluorescent) LNs and pathological (*ex vivo*) positivity to identify malignant cells.

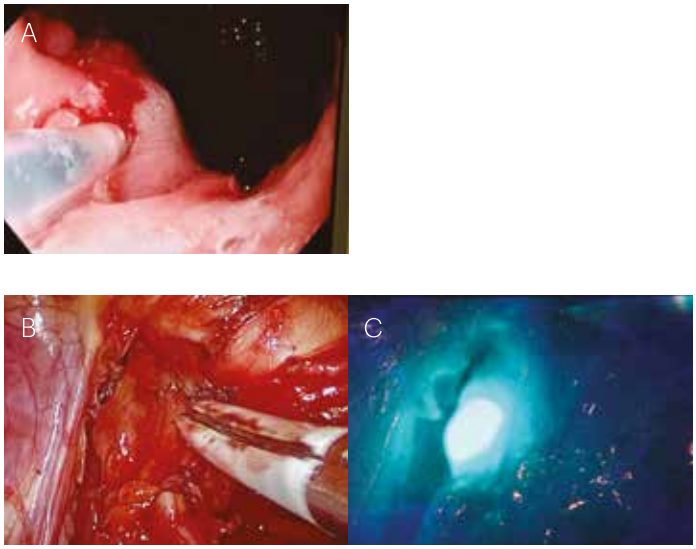


Figure 1: Indocyanine green is injected peri-tumourally endoscopically (a). (b) Thoracoscopy: is this a draining LN or fat? (c) In fluorescence mode, a draining LN that needs to be removed is identified.

Surgery commences with a neck dissection to identify the recurrent laryngeal nerve (RLN, Figure 2a) and implantation of a nerve stimulator for nerve mapping and continuous monitoring of the RLN during the thoracoscopic upper oesophageal dissection (Figure 2b, c). Continuous monitoring of the RLN allows for a more aggressive nodal dissection. Then, video-assisted thoracoscopic surgery is done via three ports plus a mini-thoracotomy through which a lung retractor and sucker may be introduced (Figure 3a, b). The patient lies on the left lateral position (Figure 3c). A Maryland LigaSure™ device is used for all thoracoscopic/laparoscopic dissections. The mean LN yield from MIOs is 45 and dissection of *ex vivo* nodal stations is usually done postoperatively by the Surgical Fellow. Thoracoscopic dissection is followed by laparoscopic gastric mobilisation, which is combined with a mini-laparotomy for gastric tubularisation (Figure 4a). This culminates with retrosternal delivery of the gastric conduit (Figure 4b) for a hand-sewn (continuous, single layered) OG neck anastomosis (Figure 4c).

Figure 2: Preoperative localisation of the RLN for the mapping and continuous monitoring of nerves (a). During thoracoscopic dissection, intraoperative utilisation of nerve monitoring by a probe identifies if a structure (b) is the nerve, a question that is answered by a monitor (c) auditorily and sinusoidally by the amplitude of the voltage gained on contact.

Figure 3 (below): Setup for thoracoscopic dissection. Three ports and mini-thoracotomy (a) for lung retraction and suction (b) with the patient on the left lateral position with Professor Law operating behind the patient (c).

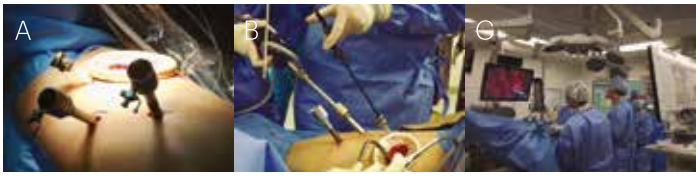


Figure 4: Tubularised conduit fashioned extracorporeally (a). Creation of a retrosternal tunnel (b) for the hand-sewn cervical anastomosis (c).

Laparoscopic gastric mobilisation is usually done by a Fellow, in conjunction with a consultant as the first assistant, with a BST/HST holding the endoscope between the legs. Following mobilisation, a mini-laparotomy is done in the epigastrium and a pyloroplasty is undertaken routinely on all gastric conduits. After preparation of the gastric conduit, ICG is administered (i.v.) by the anaesthetist and fluorescence imaging utilised via a SPY™ laser imaging system (Novodaq) to visualise the viability of the conduit before oesophagogastric anastomosis (ICG angiography, Figure 5a). This advanced fluorescence imaging modality enhances the ability to visualise and objectively assess conduit perfusion intraoperatively in real-time (Figure 5b). This provides clear demonstration of non-viable areas on the conduit that could otherwise have been incorporated into the OG anastomosis. SPY has been used for fluorescence imaging at QMH for the past 2 years and has revolutionised their practice.

Figure 5: Assessment of the viability of the gastric conduit (a). External view of the gastric conduit during laser fluorescence imaging (b) showing live images of the gastric conduit with a zone of perfusion demarcation laterally.

A retrosternal route is preferred because Professor Law has noted a reduction in the prevalence of delayed gastric emptying in comparison with conduits in the orthotopic position. He does not use a feeding jejunostomy as a 'safety net'. This is likely due to their anastomoses being in the neck and so a leak here is less morbid and easier to manage than a leak from an intrathoracic anastomosis (although this remains controversial because there is evidence that a cervical anastomosis often comes to lie in the upper part of the thoracic cavity). A single, easily-portable 'Blake' basal sump drain on free drainage is used, which includes a suction pump if required (Figure 6). Professor Law does not feel it is necessary to insert a prophylactic apical drain for potential pneumothoraces.

The single Blake drain is very light and flexible, with a reduced calibre, and does not need to be connected to an underwater seal. Consequently, patients may carry it around easily and it is more comfortable and less cumbersome than conventional intercostal drains with underwater seals. Blake drains can be used as effectively as conventional drains for air and fluid within the pleural cavity.

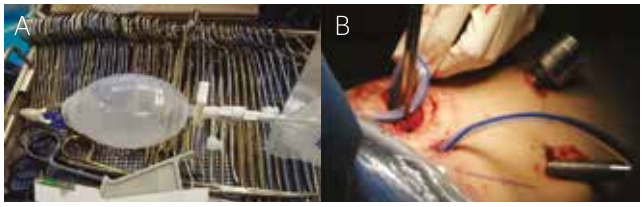


Figure 6: A Blake thoracostomy drain (a) *in situ* (b).

Post-oesophagectomy protocol

QMH has a postoperative protocol for oesophageal resections (MIO or open). Day 0 involves a stay in the ITU, allowing sips on the evening of surgery and informing the family of the outcome. On day 1, the patient is moved to the ward and sits out on a chair. Routine bronchoscopy is done to check vocal cords and sputum suctioning is undertaken. A chest radiograph is taken to check for gastric dilatation. The nasogastric tube is removed. Water is allowed on day 2 and all patients must now walk. Soup is allowed on day 4 and free fluids on day 5 if Speech and Language Therapist assessment is satisfactory. Hospital discharge is on day 7–8.

Laparoscopic gastrectomies

Although distal gastrectomies are more common in HK, a total gastrectomy is discussed here because it is the more complicated procedure.

Five ports and a Nathanson liver retractor are used at QMH (Figure 7a). ICG is injected peri-tumourally endoscopically immediately before surgery for LN recognition intraoperatively. Peritoneal cytology is undertaken immediately before dissection. A Maryland LigaSure is used for dissection. Following a D2 dissection (Figure 7b), the oesophagus is divided with an Endo-GIA™ stapler, then an OrVil™ circular stapler is passed into the oesophagus (delivered via a long tube) while a CEEA™ circular stapler is inserted into the jejunum via a mini-laparotomy in the epigastrium. A circumferential tumour occupying the entire stomach is shown in Figure 7c.

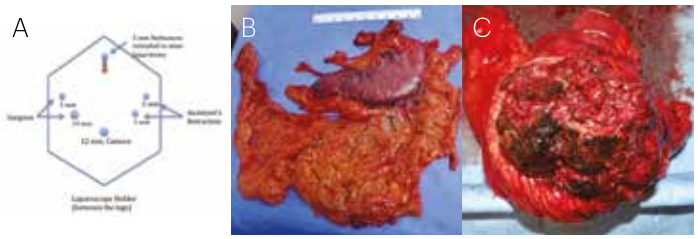


Figure 7: Standard port placements for laparoscopic gastric mobilisations/resections in HK (a). Total-gastrectomy specimen (b). A circumferential cancer occupying the entire stomach (c).

The protocol after a laparoscopic gastrectomy protocol involves allowing sips and medications on day 0. Clear fluids are allowed on day 1. Free fluids are allowed on day 2 and drinks must be consumed on this day. Hospital discharge is on day 3.

ACKNOWLEDGEMENTS
I am grateful to the Royal College of Surgeons of Edinburgh and the Association of Upper GI Surgeons for making this Fellowship possible.

Travelling Fellowship Report

Report for the Sir James Fraser Travelling Fellowship in General Surgery 2017

Adam E Frampton
ST6 General and HPB Surgery, HPB Surgical Unit, Hammersmith Hospital, Imperial College, London
October 2017

It was my privilege to receive the Sir James Fraser Travelling Fellowship 2017 to visit the Surgical Clinic at Heidelberg University Hospital in October 2017. Heidelberg is one of the largest tertiary referral centres for HPB surgery in Germany and Europe. The surgeons there undertake ≈700 pancreatic resections, 100 liver transplants, and 300 liver resections per year. Under the leadership of Professor and Chairman Markus Büchler, the unit has become the European Pancreatic Centre and is world-renowned for the surgical management of malignant and benign pancreatic diseases. It is one of the highest-volume pancreatic surgical centres in the world. The Department of Professor Büchler has also carried out important clinical trials in HPB surgery as well as extensive basic-science research into pancreatic cancer.

When I arrived at the clinic, I was greeted by Professor Alexis Ulrich (Vice Chairman), who explained the day-to-day timetable and allowed me to have free rein throughout the operating theatres and various meetings (daily handover, tumour boards, research/laboratory meetings). Professor Ulrich explained the German hierarchy and model of surgical training, and the team approach that the clinic uses. He and Professor Büchler assign operative cases each day to the oberärzte (consultant surgeons that are doing specialist work in the department). The oberärzte operate 5 days a week, whereas a separate team supervised by a senior surgeon has primary responsibility for the day-to-day management and discharge of postoperative patients. Professor Ulrich explained that he observes his more junior consultants carefully and allows them to develop their skills, while he is always available to help if they need assistance. He also told me that most surgeons in the clinic have done some form of research, and several have spent extra time in the USA doing post-doctoral research, including himself.

I attended the daily early-morning handover meetings in which the junior doctors from the wards reported problems from the previous day and overnight. The On-call team then presented the surgical referrals and admissions. Immediately after this meeting everyone went to the operating theatres, where the anaesthetic teams were preparing patients for surgery. Each day there was a wide range of procedures listed in the 17 operating theatres: at least 2–3 pancreatic resections (open and robotic), as well as resections for sarcoma, liver, oesophagogastric and colorectal cancers. The surgeons in the clinic are expected to operate throughout the gastrointestinal tract even though they specialise in HPB surgery. The senior surgeons also do liver transplantation. Professor Büchler showed me around the operating rooms and highlighted the most interesting pancreatic and hepatobiliary procedures, ensuring that the operating surgeon showed me the crucial steps and instructing the medical photographer to take pictures for me. Professor Büchler and his team have developed an approach for the radical resection of locally advanced pancreatic cancer that they call the 'triangle operation'. This strategy comprises radical resection of the tumour by sharp dissection along the coeliac axis and superior mesenteric artery, with complete dissection of all soft tissue between these vessels and the portal vein, along with an extensive lymphadenectomy, thereby allowing clarification of vascular infiltration (Figure 1).

My logbook in Heidelberg was incredible. Over 2 weeks, I was able to observe and, in most cases, I scrubbed in and assisted with, 13 pancreatic resections and a further 7 major procedures, including excision of a leiomyosarcoma with right nephrectomy and vena-cava resection; and an extended left hepatectomy for a massive liver haemangioma. I also went on-call and assisted with a subtotal gastrectomy for a necrotic

stomach following caustic ingestion; and a repair of an aorto-duodenal fistula with re-implantation of visceral arteries and endovascular aneurysm repair.

Aside from the clinical work, I attended basic-science laboratory meetings run by Dr Thomas Schmidt and Professor Martin Schneider. I listened to work-in-progress presentations and contributed to the discussions. Subsequently, I have established a research collaboration with Professor Oliver Strobel looking into the molecular mechanisms in pancreatic cancer.

I would like to thank Mrs Cathy McCartney for her administrative support and the Royal College of Surgeons of Edinburgh for the award.

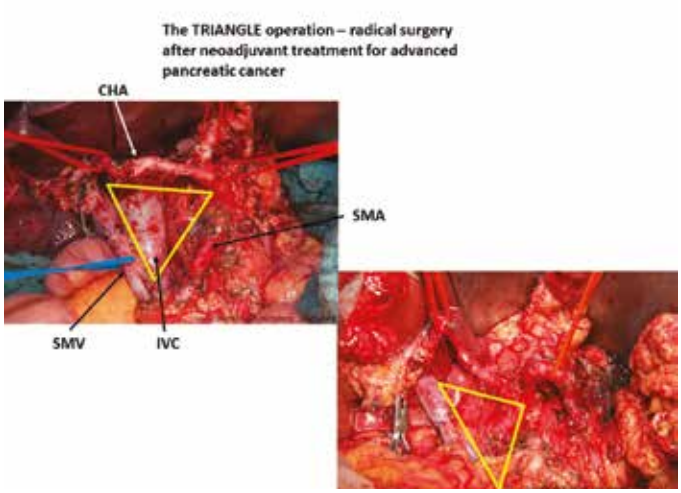


Figure 1: TRIANGLE procedure for locally advanced pancreatic cancer. CHA, common hepatic artery; SMA, superior mesenteric artery; SMV, superior mesenteric vein; IVA, inferior vena cava.

Ophthalmology Grant Report

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

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
1 June 2015 to 31 May 2016

LAY SUMMARY

Millions of people worldwide await corneal transplants. To overcome scarcity in usable donor corneas, approaches to replace corneas with 'corneal equivalents' that have similar composition and functions are ongoing. Promising strategies include transplanting corneas with soft-tissue hydrogels that support the regrowth of original corneal layers over time and restore vision. Critical limitations of such approaches are host immune responses that seek to protect the host but end by limiting transplant functionality by 'walling it off' from the rest of the eye, akin to the formation of a scab in response to a splinter. Immune responses are complex processes driven by potent dendritic cells (DCs) that arrive at hydrogel transplants and drive effector cells to accept or reject the foreign body. If DCs 'see' something as dangerous, the body expels it. If DCs ignore the foreign object, the body allows it to remain and function. Our research focuses on the heart of this process by studying DC responses to different hydrogel corneas and predicting which are likely to succeed in mouse eyes (animal model similar to human eyes) and, ultimately, in the clinic. The long-term goal is to provide accessible and affordable alternatives for patients with corneal blindness.

GRANT REPORT

(A) CLINICAL AND SCIENTIFIC SIGNIFICANCE OF ADVANCES MADE

Background

Recombinant human collagen (RHC) III hydrogels are promising corneal equivalents in animal and clinical studies[1]. Cross-species transplantation into murine hosts results in low-grade antibody responses against porcine collagen hydrogels. DCs link innate and adaptive immunity; recognition as self *versus* non-self triggers tolerance or maturation of DCs, resulting in immune quiescence or activation[2]. DC responses to corneal transplants may determine the balance between host immunity *versus* tolerance and influence the fate of corneal grafts[3]. Examining DC responses to differentially crosslinked hydrogels *in vitro* may predict their immune acceptance *in vivo* after implantation in murine corneas. Hydrogels that minimally activate DC may be beneficial in corneal regenerative applications.

Results summary – Study 1

Murine bone marrow-derived DCs were cultured on differentially crosslinked RHCIII hydrogels for 24 h to examine the effects of collagen crosslinking on DC maturation and compare against natural collagenous matrices. DCs were utilised *in vitro* to assess the adjuvant effects of differentially crosslinked RHCIII hydrogels (representing artificial corneas) and compared with tolerogenic de-cellularised corneal stroma and gel-based extracts of the basement membrane (representing natural matrices) (Figure 1).

- **I: Natural matrices**
  - Matrigel™ (collagen IV)
  - Corneal stroma (collagen I, V, VII)
- **II: Hydrogels**
  - **EDC-NHS:** *N-(3-dimethylaminopropyl)-N'-ethylcarbodiimide;N-hydroxysuccinimide*
  - **MPC:** *2-methacryloyloxyethyl phosphorylcholine*
  - **CMP:** collagen mimetic peptide [(Pro-Lys-Gly)4(Pro-Hyp-Gly)4(Asp-Hyp-Gly)4]
  - **CMC-NHS:** *N-cyclohexyl-N'-(2-morpholinoethyl) carbodiimidemetho-p-toluenesulphonate*
- **III: Hydrogel constituents**
  - Gels (collagen I, III)
  - Polyethylene glycol (PEG)
  - CMP-PEG

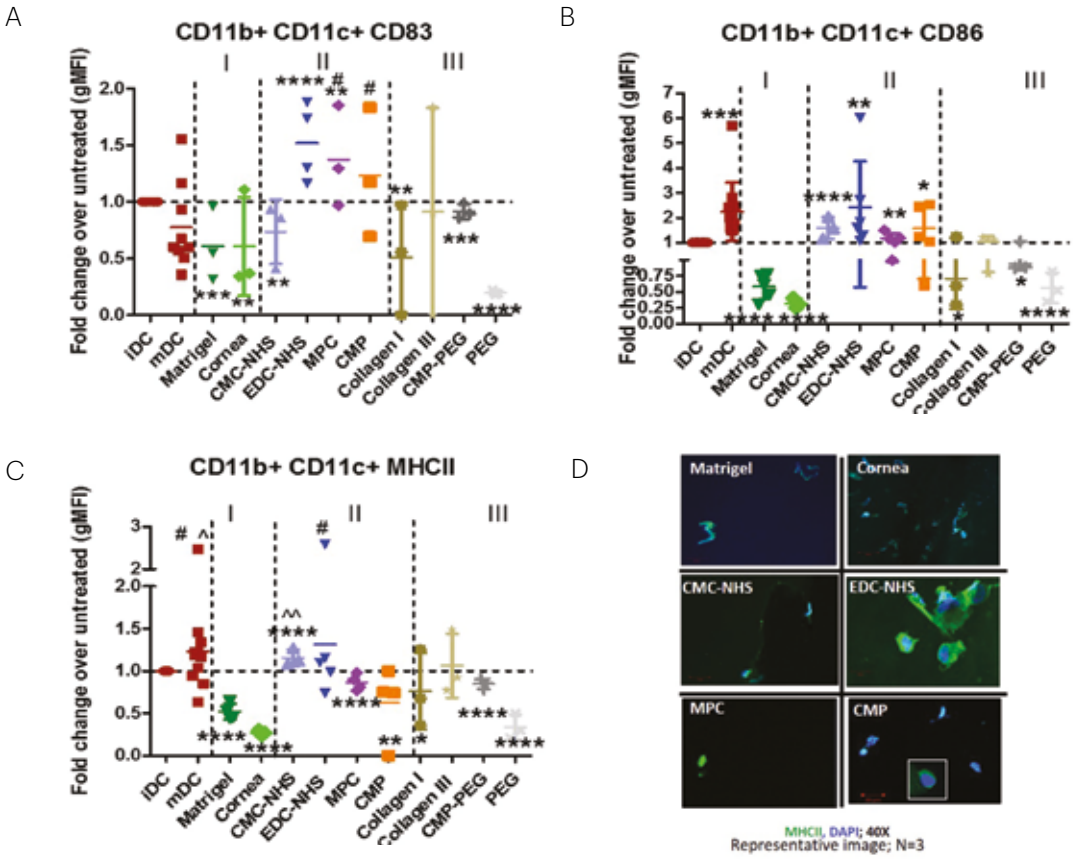


Figure 1: Differential activation of DCs by natural matrices, hydrogels or hydrogel constituents (24 h). Expression of a marker of DC maturation for CD83 (A), CD86 (B) or MHCII (C) for CD11b+ CD11c+ cells. Mean ± SD; N = 3–8, unpaired *t*-test; *different from iDC; p≤0.05, one-way ANOVA with Tukey post-test, #from PEG, ^ from cornea; * p≤0.05, **p≤0.01, ***p≤0.001, ****p<0.0001. MHCII expression for DCs infiltrated inside natural materials or hydrogels (N = 3) (D).

Matrigel™ or decellularised corneal stroma downregulated expression of CD83 (Figure 1A), CD86 (Figure 1B) or major histocompatibility complex (MHC)II (Figure 1C) on DCs *versus* immature dendritic cells (iDCs), indicating that natural matrices had tolerogenic effects. Hydrogels were intermediate (CMC-NHS, EDC-NHS) or low activators of DCs (MPC, CMP) (Figure 1A–C). CMP hydrogels downregulated expression of MHCII to comparable levels as natural matrices *versus* iDC controls and were least activating of DCs due to constituent tolerogenic PEG backbones (Figure 1C). Increased expression of MHCII indicative of DC maturation was displayed on DCs cultured on activating EDC-NHS hydrogels as opposed to counterparts on CMP hydrogels or as DC debris for Matrigel or corneas (Figure 1D).

We hypothesised that DCs were minimally activated on certain biomaterials due to increased tolerogenic apoptosis of DCs. We observed decreased numbers of live DCs and increased numbers of dead DCs cultured on natural matrices and increased numbers of late-apoptotic DCs on low activating CMP hydrogels *versus* other gels at 24 h (Figure 2A–D). Percentages of live, early-apoptotic, late-apoptotic or dead DCs are shown for all biomaterials as stacked bar graphs (Figure 2E). We examined the relative contributions of individual hydrogel constituents on DC maturation to identify gel components that increased or mitigated DC activation. Higher percentages of dead DCs cultured on non-diluted hydrophilic anti-fouling PEG (≈90%) (data not shown), a constituent of CMP hydrogels, were noted. Across biomaterials, we observed a direct correlation between live DCs and DC maturation (data not shown), highlighting an association between apoptosis and tolerisation of DCs.

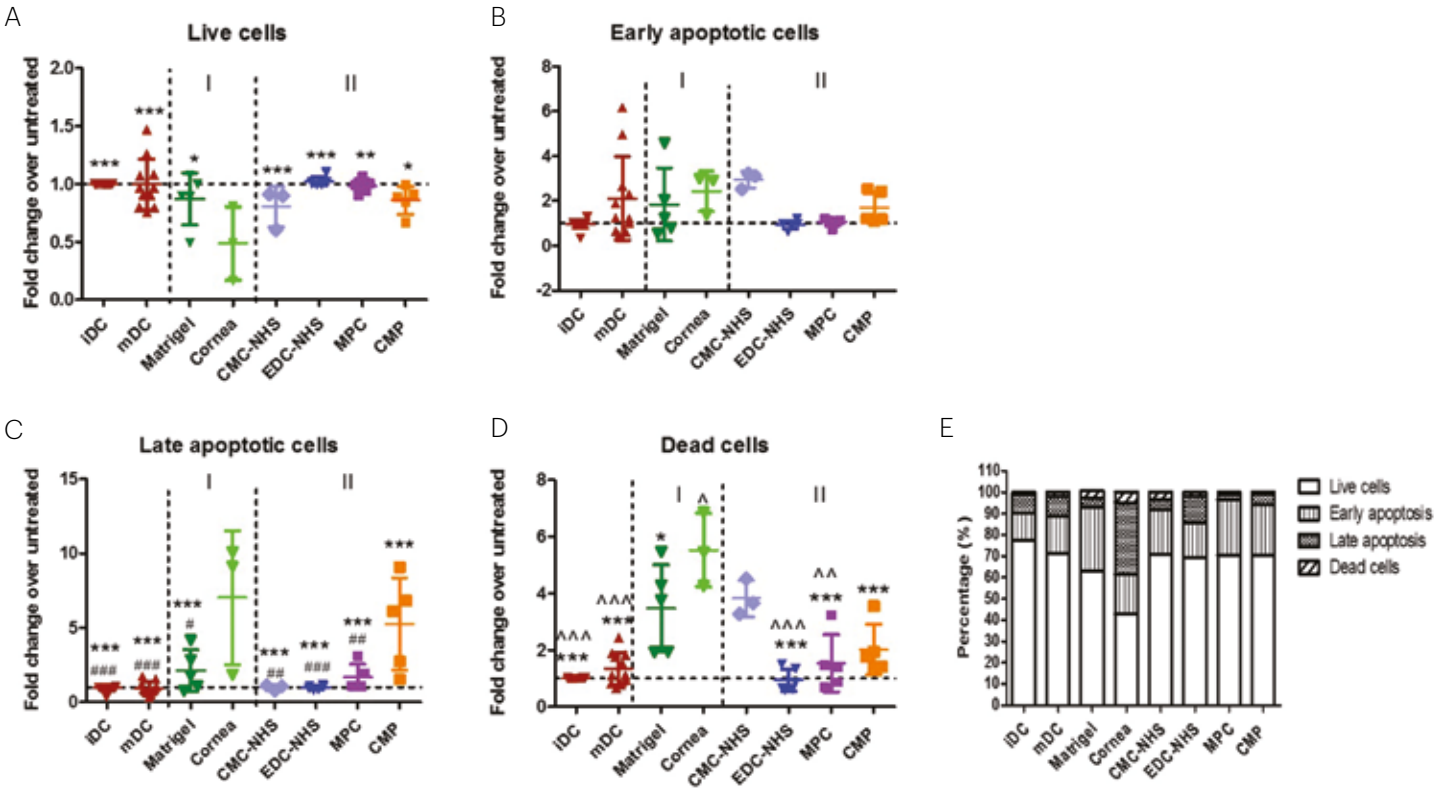


Figure 2: Increased apoptosis of CD11b+ CD11c+ DCs on minimally activating natural matrices or hydrogels at 24 h. Live (A), early-apoptotic (B), late-apoptotic (C) or dead DCs (D) on hydrogels or matrices. Mean ± SD; N = 3–8, one-way ANOVA with Tukey's post-test, *different from cornea; *, ^ different from Matrigel; #different from CMP: p≤0.05, **p≤ 0.01, ***p≤ 0.001, ****p<0.0001. Distribution of DCs at different stages of apoptosis (E).

For identifying receptor-ligand apoptotic triggers, we focused on a leucocyte-associated immunoglobulin-like receptor (LAIR)-1, an immunoinhibitory, collagen-binding receptor expressed by DCs (Figure 3A). LAIR-1 engagement elicits increased activity of the catabolic enzyme indoleamine-2,3 dioxygenase (IDO) and increased DC apoptosis. Increased expression of LAIR-1 was measured following DC culture on Matrigel versus iDC controls, and demonstrated a novel mechanism of LAIR-1-mediated DC homeostasis in the basement membrane (Figure 3A), also suggested by a non-significant upregulation of mRNA levels of IDO for Matrigel (Figure 3D).

Among hydrogels, CMC-NHS, EDC-NHS or MPC triggered increased expression of LAIR-1, suggesting a similar mechanism (Figure 3A). For minimally activating CMP hydrogels, LAIR-1 expression on DCs was comparable with that of iDCs (Figure 4A) due to the additive effects of increased or decreased LAIR-1 levels for CMP or PEG constituents, respectively. This demonstrated that DC apoptosis was triggered by chemical effects, and was confirmed by non-detectable IDO mRNA levels for PEG alone (Figure 3D). A highly significant inverse correlation was observed between LAIR-1 expression and live DCs (Figure 3B), underscoring a role for LAIR-1 in causing DC apoptosis.

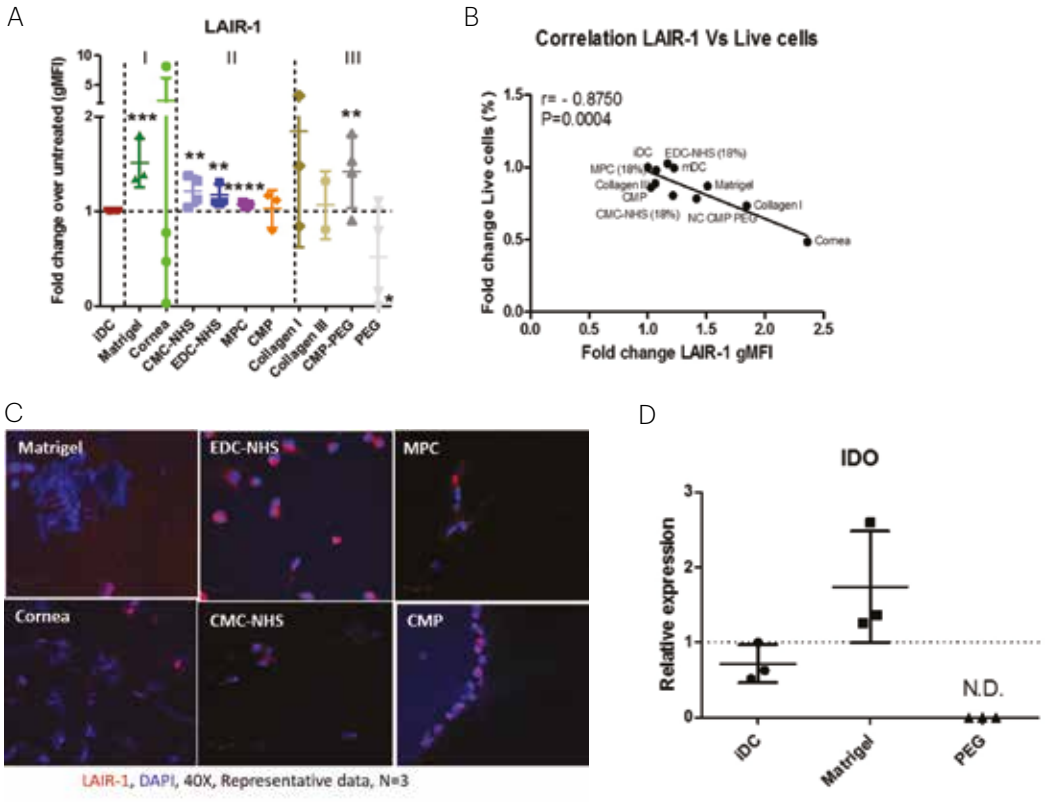


Figure 3: Increased expression of LAIR-1 for CD11b+ CD11c+ DCs cultured on minimally activating Matrigel, but not for counterparts on tolerising CMP hydrogels. LAIR-1 expression (A); mean ± SD; N = 3, unpaired *t*-test, *different from iDC, *p≤0.05, **p≤0.01, ***p≤ 0.001, ****p<0.0001. Negative correlation between LAIR-1 expression and live cells for DCs cultured on different biomaterials (B); mean ± SD; N = 3, r: Pearson's coefficient −1 ≤0 ≤1. LAIR-1 expression for DCs infiltrated into natural materials or hydrogels (N = 3) (C). IDO mRNA levels for DCs cultured on Matrigel or PEG; mean ± SD, N = 3 (D).

A DC migration assay was done (pore transwell = 5 µm) with hydrogels on membranes, DCs in upper chambers, and media alone in lower chambers (3 h, 37°C) (Figure 4A). Matrigel supported minimal DC migration as a percentage of total thickness (≈50%) (Figure 4B) whereas corneas or CMP hydrogels allowed complete cellular penetration (≈100%) (Figure 4B). At leading edges, characteristic migratory DCs were observed for CMC-NHS (Figure 4C) and quantified as invasion/migration ratios over controls in (Figure 4E), confirming low penetration levels for Matrigel and comparable levels for hydrogels. Most cells adhered to the membranes beneath biomaterials (Figure 4D), indicating that they had migrated along and around the hydrogels and promoted the formation of retro-hydrogel membranes that obstruct vision in murine corneal transplants (Study 3)[3]. Fewest cells were present in the lower chambers of Matrigel, thereby confirming least penetrability; comparable events were measured for different hydrogels (Figure 4F). Together, CMP hydrogels or corneas exhibited the characteristics of maximum cell penetration, whereas Matrigel exhibited the minimum.

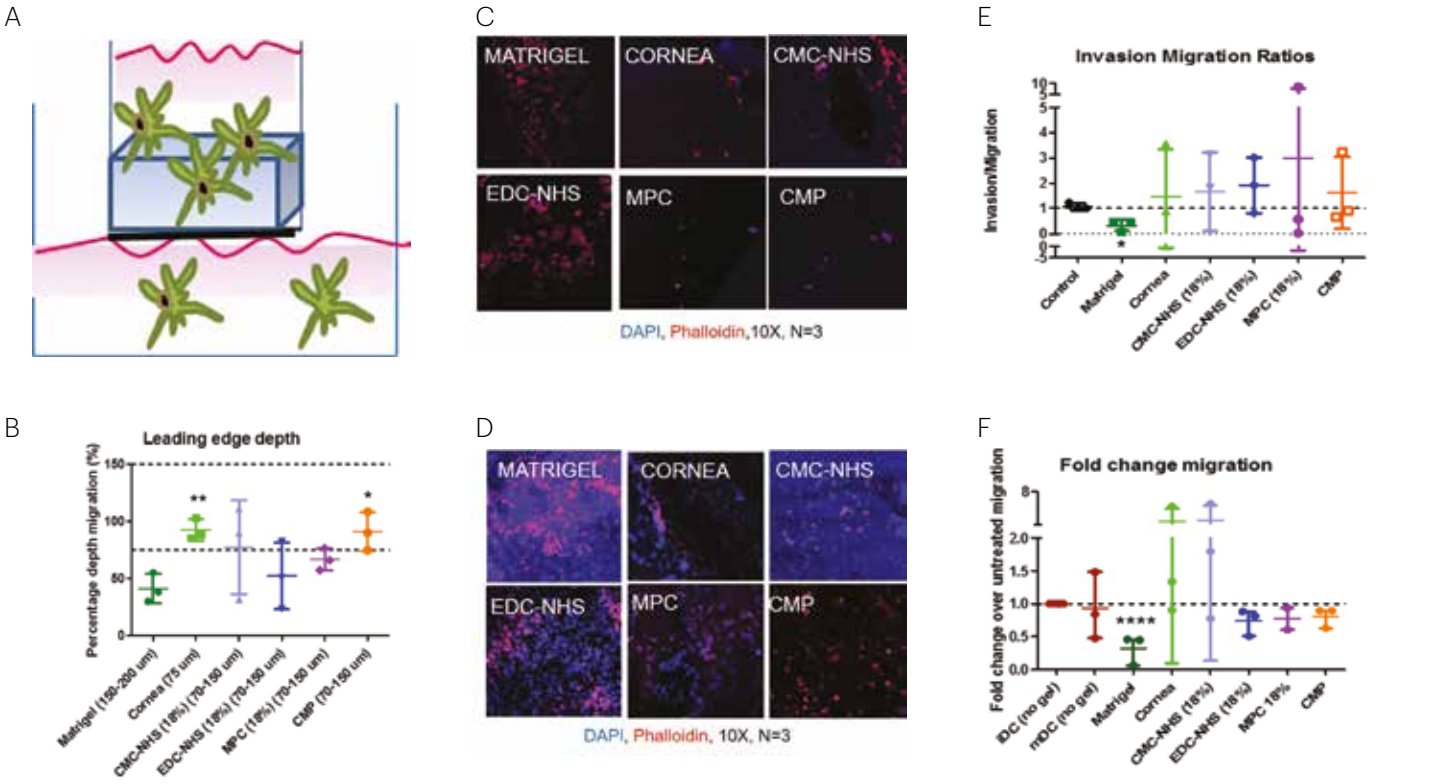


Figure 4: Migration assay (schematic) (A). Depth of the leading edge across biomaterials (B); mean ± SD; N = 3, unpaired *t*-test, *different from iDC, *p≤0.05. Cells at leading edges (C); N = 3, (E); mean ± SD; N = 3, unpaired *t*-test, *different from iDC, *p≤0.05. Cells adherent to membranes beneath gels (D); N = 3. Migratory cell events in lower chambers (F); mean ± SD; N = 3, unpaired *t*-test, *different from iDC; ****p<0.0001.

Results summary – Study 2 (ongoing)

The relative localisation patterns of recruited bone marrow-derived dendritic cells (BMDCs) *versus* resident corneal DCs are being characterised under different corneal pathological or transplantation scenarios. Specifically, the differential presence of resident *versus* recruited corneal CD11b+ CD11c+ immune cells are being assessed in corneal epithelia, stromal edges/centres or in endothelia. The objective is to gain an understanding of the conditions under which BMDCs are allowed to infiltrate stroma and their subsequent interactions with resident immune cells.

Enhanced green fluorescent protein (EGFP) DCs were cultured on non-EGFP corneas for 24 h and the following scenarios where BMDCs may be recruited were modelled:

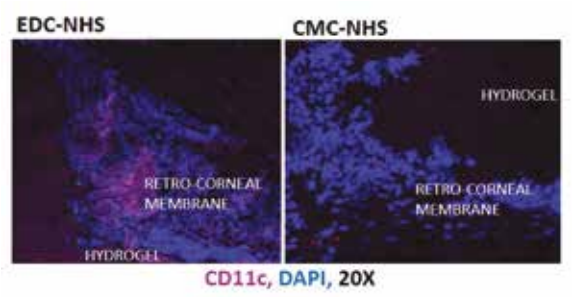
- Healthy corneas with/without epithelium exposed to localised injury
- Inflamed (lipopolysaccharide-treated) corneas exposed to re-insult
- 'Naturally' de-cellularised corneal transplants (CD11c+ egress from transplants in media).

Results summary – Study 3 (ongoing)

Murine corneas underwent penetrating keratoplasties with EDC-NHS or CMC-NHS hydrogels and were assessed 22 days post-transplantation (pt)[3]. Hydrogels supported regenerative remodelling but triggered the formation of vision-obstructing retro-hydrogel membranes[3]. Both types of hydrogels became opaque, but the underlying mechanisms were dissimilar. Comparable extents of α-smooth muscle actin in wound healing or cytokeratin-12 in re-epithelialised membranes were observed (data not shown). CD11c+ DC were present around hydrogels 22 days pt in EDC-NHS-transplanted eyes (Figure 5A) but they could not be visualised with CMC-NHS counterparts (Figure 5B), possibly due to varying cellular kinetics. DCs were visualised within transplanted CMP hydrogels (6 and 24 h) (data not shown), suggesting that DCs have crucial roles in shaping immune responses.

Differential amounts of tenascin C (which is involved in epithelial-to-mesenchymal transition and produced by

A



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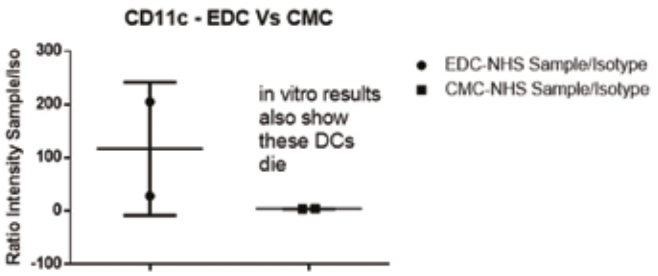

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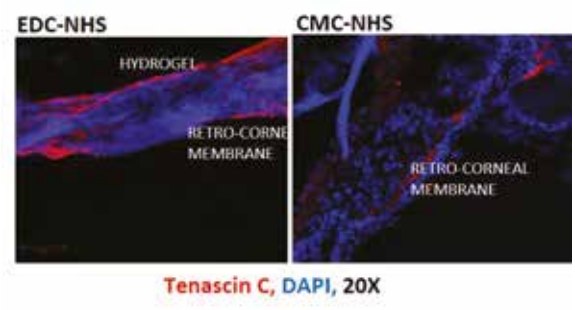

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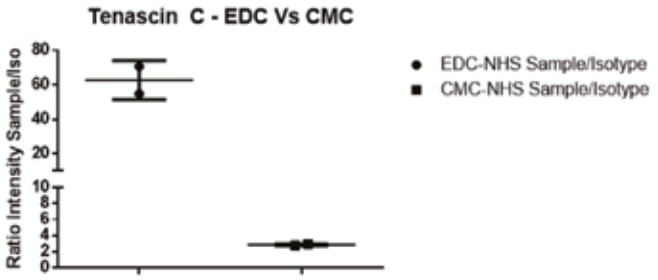


Figure 5: DCs have a role in host responses to RHCIII hydrogel transplants of murine corneas. CD11c+ DCs (A) or tenascin C (B) were present around EDC-NHS-transplanted corneas and to reduced extents for CMC-NHS counterparts (N = 4; unpaired *t*-test: p≤0.05).

fibroblasts during early wound healing) were detected. EDC-NHS hydrogels, which supported DC recruitment, were associated with higher levels of tenascin C (Figure 5C) *versus* CMC-NHS-transplanted eyes (5D). CMC-NHS hydrogels (Figure 5D) may trigger increased DC apoptosis *in vivo*, resulting in their relative absence compared with EDC-NHS hydrogels that promote DC survival (Figure 2). Do hydrogel-associated DCs then regulate secretion of tenascin C by fibroblasts? This study is important because it highlights a potential novel association between DCs and wound healing that is being investigated *via* DC-fibroblast co-cultures (data not shown).

Conclusions

Natural matrices such as corneal stroma and basement membrane differentially modulate DC activation compared with type-I- and III-based collagen matrices which are the 'normal' matrices through which DCs migrate from tissues to lymph nodes during the adaptive immune response. 'Corneal equivalent' hydrogel matrices also modify DC behaviour differentially, including effects on the migration, infiltration, maturation and survival of DCs, all of which impact on host responses that are crucial for promoting graft functionality in grafted/implanted corneas.

Scientific importance

Major advances were:

- Demonstration of roles for DCs in mediating host adaptive responses to hydrogel transplants and possible roles in wound healing.
- Establishment of an *in vitro* screening system enabling rapid, high-throughput and inexpensive prediction of successful hydrogel candidates for artificial corneas, based on their interactions with DCs.

Clinical importance

This study will generate criteria for optimal hydrogel design towards supporting wound healing, tissue integration and sustained functionality for pre-clinical/clinical applications.

(B) PROBLEMS ENCOUNTERED AND STEPS TAKEN TO OVERCOME THEM

Scientific

- Kynurenine, an indicator of IDO activity, was quantified using a chemical reaction on precipitated proteins. However, the assay was not taken forward due to technical problems and was replaced by quantitative polymerase chain reaction (qPCR) to measure IDO levels.

Logistical

- Minor issues from year 1 were resolved.

References

1. Fagerholm P, Lagali NS, Ong JA, et al. Stable corneal regeneration four years after implantation of a cell-free recombinant human collagen scaffold. *Biomaterials* 2014;35:2420–2427.
2. Steinman R. Decisions about dendritic cells: past, present, and future. *Annu Rev Immunol* 2012;30:1–22.
3. Ahn J, Kuffova L, Merrett K, et al. Crosslinked collagen hydrogels as corneal implants. *Acta Biomater* 2013;9:7796–7805.

(C) COLLABORATIONS ESTABLISHED

With Professor May Griffith, Professor of Regenerative Medicine & Director, Integrative Regenerative Medicine Centre, Linköping University.

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

Publications

- Shankar SP, Griffith M, Forrester JV, et al. Dendritic cells and the extracellular matrix: a challenge for maintaining tolerance/homeostasis. *World J Immunol* 2015;5:113–130.
- Shankar SP, et al. *In vitro* responses of dendritic cells to hydrogels in artificial corneas [in preparation].
- Shankar SP, et al. Dendritic cells and tenascin C in hydrogel corneal transplants [in preparation].

Funding

- Royal College of Surgeons of Edinburgh, Major Project Ophthalmology Grant, 2016–2017 (£49,971).

(E) ACKNOWLEDGEMENTS

We acknowledge the Royal College of Surgeons of Edinburgh and Royal Blind for funding received during 2014–2016. We thank the confocal microscopy, flow cytometry, qPCR facilities and Medical Research Facility staff at the University of Aberdeen for technical assistance.

Ophthalmology Grant Report

Optimising gene therapy treatments for dominant retinitis pigmentosa

Professor Robert E MacLaren
Nuffield Laboratory of Ophthalmology, University of Oxford
1 October 2015 to 30 September 2016

LAY SUMMARY

Most incurable causes of blindness in the developed world affect the light-sensing cells known as 'photoreceptors', which line the back of the eye in a dot-like array similar to the pixels of a computer screen. The structure that contains the photoreceptor cells and the other nerve cells that form connections between them is known as the 'retina'. The latter connects through the optic nerve to the brain, where the image is perceived. Retinitis pigmentosa (RP) is a genetic disease that affects photoreceptors and causes blindness. Most RP cases are caused by a faulty gene which makes a defective form of an important protein known as 'rhodopsin' (RHO). Production of defective RHO causes photoreceptor cells to die. Retinal gene therapy is a new technology which allows correction of faulty genes. Photoreceptors have two copies of the RHO gene, so the ratio of normal genes to faulty genes is usually 1:1. We wished to develop a new method of gene therapy to augment expression of the normal RHO gene so that the toxic effects of the faulty gene are diluted significantly. We are assessing the efficacy of this gene therapy in human tissue and mice.

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 2 years, we have expanded our trials on retinal gene therapy to the USA, Canada and Germany using the same vector developed in part through one of the previous grants from the Royal College of Surgeons of Edinburgh. Together with the Wellcome Trust, the University of Oxford has formed NightstaRx Limited, a company specialising in retinal gene therapy. NightstaRx has attracted £40 million in venture capital funding, which will be used to run an international, multicentre, pivotal 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.

Background to the current project

The purpose of this project is to develop a treatment for dominantly inherited RP caused by a defective copy of the RHO gene. We aim to develop an adeno-associated viral (AAV) vector which can express a healthy copy of the RHO gene to a sufficient level in photoreceptors so as to reduce the proportion of mutant-to-normal RHO mRNA in patients suffering from dominantly inherited RP.

Specific objectives of the current project

This first year of a possible 3-year project has three specific aims:

- Compare the efficiency of a self-complementary vector, in which a single strand of DNA is folded over into a double-stranded loop, with that of three alternative single-stranded vectors.
- Validate the human RHO promoter in RHO-null mice.
- Determine the effect of RHO supplementation on retinal degeneration in a P23H-RHO knock-in model of dominant RP in mice.

Results obtained

Four RHO-expressing AAVs were manufactured: a self-complementary RHO vector (SC-AAV), a single-stranded RHO vector (RHO-AAV), a single-stranded RHO vector with the woodchuck hepatitis virus post-transcriptional

regulatory element (WPRE-AAV), and a single-stranded RHO vector with WPRE and an additional exon/intron splice site from the chicken beta-actin promoter (Ex/Int-AAV). These four vectors induced expression of RHO protein when tested in dissociated retinal cells from the RHO knock-out mouse which lacks native RHO (Figure 1).

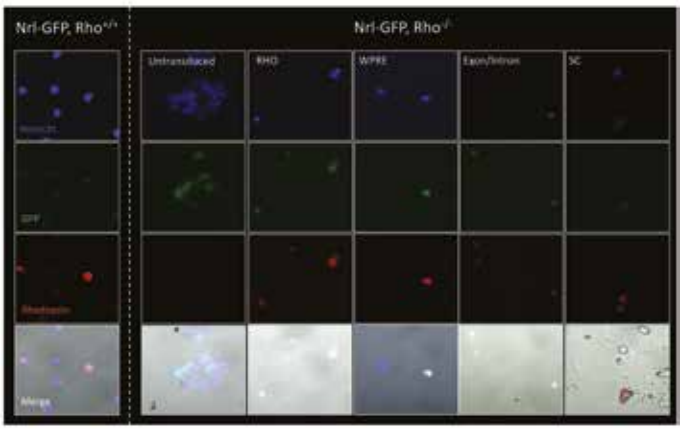


Figure 1: RHO expression in AAV-transduced dissociated cells. Retinas from RHO knock-out mice with cells of the rod lineage labelled with green-fluorescent protein (GFP) were dissociated and transduced with the four rhodopsin AAVs. All cell nuclei were counterstained with Hoescht (blue). GFP co-localised with RHO stain (red) in transduced but not in untransduced RHO knockout cells.

Comparison of mRNA levels using this assay showed significantly greater expression induced by self-complementary *versus* single-stranded vectors. Subretinal delivery of each of the four vectors in RHO knock-out mice led to expression of RHO protein that formed multimeric complexes akin to those found in wild-type animals (Figure 2).

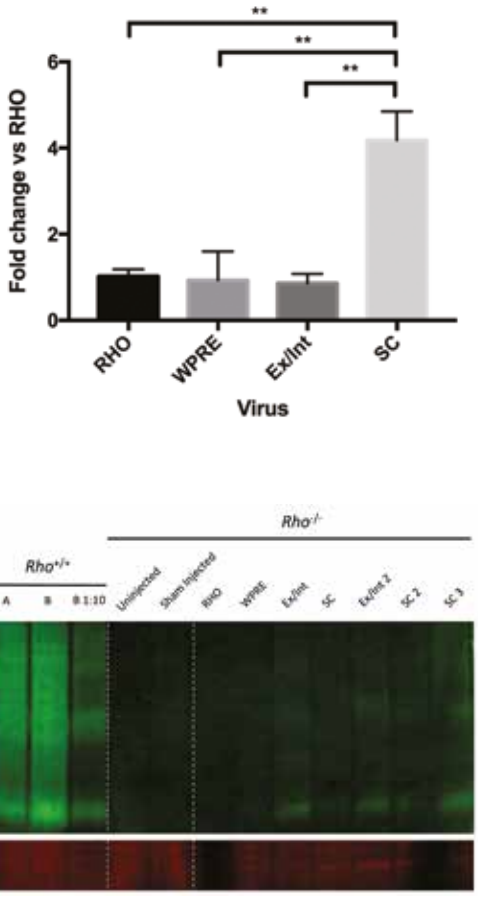


Figure 2: The graph plots RHO mRNA levels derived from AAV-transduced RHO knock-out dissociated retinal cells for the four experimental viruses all normalised to single-stranded RHO-AAV. The western blot illustrates the similar multimeric complexes formed by native RHO in uninjected wild-type and RHO AAV-injected RHO knock-out retinas.

P23H RP mice received unilateral injections of self-complementary RHO AAV at one of two doses or normal saline (sham). In all cases the contralateral eye was left as an untreated control. Preliminary data from these cohorts suggested a promising early-rescue effect in eyes receiving high-dose vector compared with those receiving low-dose vector or sham. This result applied to retinal structure (as recorded by mean grey value (a measure of surviving fluorescence in green fluorescent protein-labelled rod cells)) and function (as measured by rod-isolating electroretinography (ERG)) (Figure 3). These cohorts of mice will be followed for 6 months to determine if the effect is sustained.

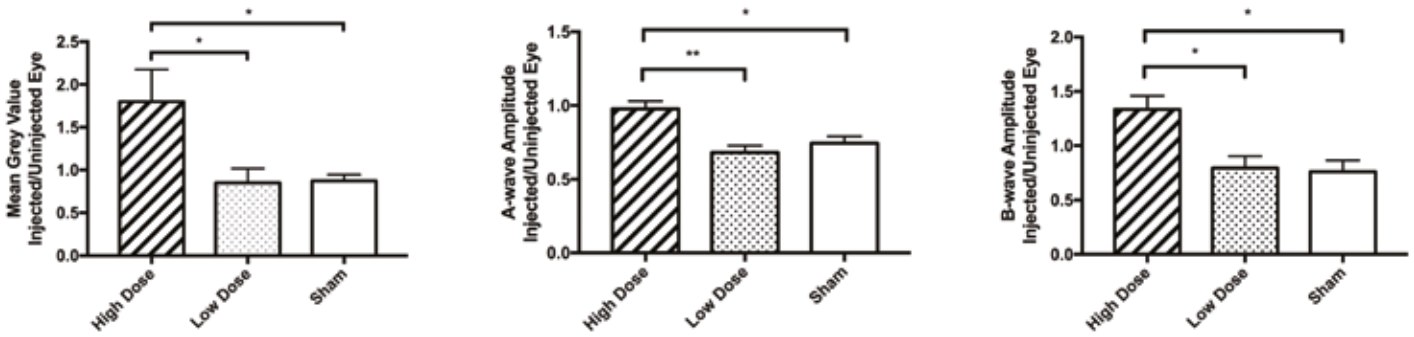


Figure 3: Evidence of early structural (left) and functional (middle and right) rescue of the P23H knock-in model of human dominant RP following subretinal delivery of high-dose self-complementary RHO AAV. *p<0.05, **p<0.01.

(B) PROBLEMS ENCOUNTERED AND STEPS TAKEN TO OVERCOME THEM

Acquiring quantitative comparative data from transduced retinal cells proved challenging owing to the small amounts of mRNA and protein obtained. Furthermore, large amounts of virus were required to obtain measurable levels of transgene expression. A comparison of mRNA levels by reverse-transcription qPCR was possible after significant optimisation of the protocol, but obtaining sufficiently high levels of protein to compare vectors by western blotting was not possible. A comparison of protein levels achievable with the four vectors will, therefore, be made *in vivo* by subretinal injections in RHO null mice. These retinas have now been harvested and western blotting will be conducted.

(C) COLLABORATIONS ESTABLISHED

An arrangement has been established between the MacLaren research team and the macaque-breeding facility at the Defence Science and Technology Laboratory at Porton Down to allow members of the team to obtain fresh retinal tissue from animals scheduled to be culled for unrelated reasons. Such tissue has allowed the *ex vivo* validation of AAV vectors in primate tissue and has proved extremely valuable.

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

Higher degrees and prizes

Dr Harry Orlans (an ophthalmologist in training who is currently enrolled as a doctoral student in the MacLaren research team) was awarded the Thomas Willis Poster Prize by the Nuffield Department of Clinical Neurosciences at the University of Oxford for the best poster presented by a first-year doctoral student. The data presented in the poster related to validation of the efficacy of WPRE in human retinal tissue which formed the basis of the design of the AAVs used in the RHO project outlined above. The research was undertaken by Dr Orlans under the supervision of Dr Alun Barnard, the post-doctoral research scientist who was supported by the Royal College of Surgeons of Edinburgh award. Since commencing his DPhil, Dr Orlans has been awarded Fellowship of the Royal College of Ophthalmologists. Furthermore, preliminary data acquired with funding from the Royal College of Surgeons of Edinburgh formed the basis of a 3-year Clinical Training Research Fellowship awarded to Dr Orlans which is jointly funded by the MRC and Fight for Sight.

Publications

- Patrício MI, Barnard AR, Orlans HO, et al. Inclusion of the woodchuck hepatitis virus posttranscriptional regulatory element enhances AAV2-driven transduction of mouse and human retina. *Mol Ther Nucleic Acids* 2017;6:198–208.
- Orlans HO, Edwards TL, De Silva SR, et al. Human retinal explant culture for *ex vivo* validation of AAV gene therapy. Book chapter in *Retinal Gene Therapy: Methods and Protocols*. 2018 [in press].

- Orlans HO, Barnard AR, MacLaren RE. Quantitative assessment of photoreceptor degeneration by confocal scanning laser ophthalmoscopy in two mouse models of retinitis pigmentosa. Abstract accepted for presentation at the Association for Research in Vision and Ophthalmology (ARVO) meeting, Baltimore, 2017.

Several scientific studies arising from previous research projects supported by the Royal College of Surgeons of Edinburgh have been published during this 1-year research project. These publications, which all acknowledged the support of the Royal College of Surgeons of Edinburgh, are:

- Barnea-Cramer AO, Wang W, Lu SJ, et al. Function of human pluripotent stem cell-derived photoreceptor progenitors in blind mice. *Sci Rep* 2016;6:29784.
- Xue K, Oldani M, Jolly JK, et al. Correlation of optical coherence tomography and autofluorescence in the outer retina and choroid of patients with choroideremia. *Invest Ophthalmol Vis Sci* 2016;57:3674–3684.
- Edwards TL, Jolly JK, Groppe M, et al. Visual acuity after retinal gene therapy for choroideremia. *N Engl J Med* 2016;374:1996–1998.

(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 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

Identification of new mechanisms and targets in retinoblastoma

Mandeep S Sagoo and Shin-Ichi Ohnuma
Institute of Ophthalmology, University College London
1 November 2015 to 31 October 2016

LAY SUMMARY

Retinoblastoma (RB) is an aggressive cancer of the primitive retina. It was one of the first cancers to have a genetic basis confirmed with mutation in the Rb1 tumour-suppressor gene. Treatment is highly specialised, with radiotherapy, chemotherapy, lasers and cryotherapy employed to destroy tumours. However, many eyes undergo enucleation to save the child's life. Progress in the diagnosis and treatment over the last 20 years has been slow and focused on new approaches to treatment delivery. Beyond mutation of the Rb1 gene, little has been discovered in terms of downstream molecular mechanisms that may help to diagnose treatment-resistant phenotypes and could lead to biologically tailored treatment strategies.

This project has been the first step in setting up translational research using human RB samples. In cell lines and knockout mice we have shown that genes other than Rb1 can cause changes in retinal architecture that allow a tumour-permissive environment. Further experiments, including whole genome sequencing, will follow from this work.

GRANT REPORT

(A) CLINICAL AND SCIENTIFIC SIGNIFICANCE OF ADVANCES MADE

The Small Leucine Rich Proteoglycans (SLRPs) are a family of 15 secreted small proteins in the extracellular matrix. Our pilot studies showed that healthy ocular tissues express high levels of osteomodulin (OMD) and proline/arginine-rich and leucine-rich repeat proteins (PRELPs) of the SLRP family but their expression is downregulated in RB. This may have a protective effect against tumour development and may explain why cancer affects the eye less frequently than other organs.

From the work supported by this grant, we have two important new pieces of information. First, we have investigated the effect of OMD or PRELP knockout on mouse retinas. Our knockout mice demonstrated a loose structure of the retina and we often observed dysplasia in retinas (Figure 1), indicating that cell–cell adhesion of retinal cells was weakened.

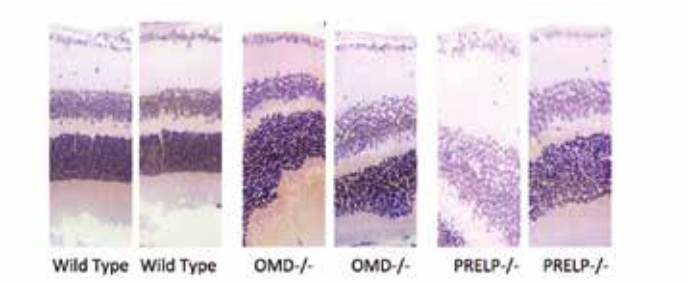


Figure 1: Retinas of wild type, OMD–/– and PRELP–/– mice

Then, we examined the effect of OMD or PRELP overexpression in the RB cell line Y79. Normally, Y79 cells float and grow in a completely anchoring-independent manner, which is a hallmark of malignant cancer cells. Interestingly, overexpression of OMD or PRELP completely changed their cellular property and all cells attached to the cell culture flask, suggesting that OMD or PRELP converted malignant cancer into a more benign phenotype (Figure 2). Importantly, the PRELP gene is located in the chromosomal region that is associated with conversion from benign retinoma to malignant RB, suggesting that OMD or PRELP might be important for malignant conversion of RB.

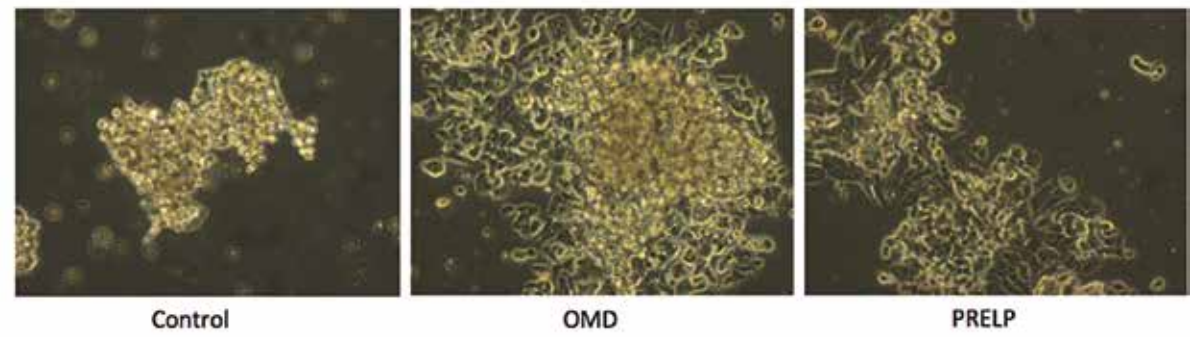


Figure 2: Effect of overexpression of OMD or PRELP in Y79 retinoblastoma floating cells

In parallel with this work, we have embarked on a genomics study in RB from fresh samples of enucleated eye tumours and archived DNA in a paediatric ocular oncology consortium. We have raised sufficient funds for a single-cell separator to be able to carry out single-cell genomics in eye cancers. We have recruited a postdoctoral research assistant with a background in cell biology and genomics. He will continue the SLRP experiments, examine the characteristics of cancer stem cells, and correlate the bioinformatics data with our experiments. For the first time, this will be done in single cells rather than homogenised tumour tissue.

(B) PROBLEMS ENCOUNTERED AND STEPS TAKEN TO OVERCOME THEM

Obtaining ethical approval for testing human samples from enucleated eyes, as well as our RB genetics archive, took 4 years. During the period of this grant we have obtained ethical approval, site approvals and have now started experiments on the genomic study in parallel with the current study. We have also undertaken immunohistochemistry and qRT-PCR looking at SLRP expression patterns and markers of cancer stem cells in RB.

Recruitment of a suitable applicant who could undertake analyses of cell biology, genomics and bioinformatics was difficult. At the third advertisement for the position we recruited an experienced postdoctoral researcher who will carry out the remaining experiments and develop his analytical skills in bioinformatics during this project.

(C) COLLABORATIONS ESTABLISHED

- Dr Zerrin Onadim, Retinoblastoma Laboratory, Royal London Hospital – Genetics input, including archived samples for analyses.
- Mr Ashwin Reddy, Consultant Ophthalmologist, Royal London Hospital – Enucleation of RB eyes for fresh tumour tissue.
- Dr Serena Nik-Zainal, Principal Investigator, Cancer Genomics, Sanger Centre, Cambridge – Whole genome sequencing.

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

We have not yet published articles from this study but hope to do so.

Mr M Sagoo was awarded the PJ Hay Medal of the North of England Ophthalmology Society for his work on eye tumours, including paediatric eye cancers.

(E) ACKNOWLEDGEMENTS

We are grateful to the Royal College of Surgeons of Edinburgh and Royal Blind for support in this project. Moorfields Eye Charity and a donation from Bluewater Energy/Sikorski/Boas allowed us to start the genomics part of our RB programme.

Ophthalmology Grant Report

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

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
1 June 2016 to 31 May 2017

LAY SUMMARY

Millions worldwide await corneal transplants. To overcome scarcity in usable donor corneas, efforts to develop 'corneal equivalents' with similar composition and functions are ongoing. Promising candidates include soft-tissue hydrogels supporting the regrowth of original corneal layers over time and restoring vision. Critical limitations are host immune responses that can result in graft rejection. These complex processes are driven by DCs that interact with the hydrogels and drive effector cells to accept or reject the gel. If DCs identify the gel as foreign (i.e., dangerous) the body expels it. If by contrast they ignore the object, the body allows it to remain and function.

In continuation of our previous research focussing on the fate of corneal grafts in mice, we aimed to verify those observations made using cell cultures. These included investigating how DCs mature or differentiate when cultured with a natural protein tissue preparation (matrix) called Matrigel™ and compared with culture with the synthetic protein hydrogel, CMC-NHS (18% collagen), which is used as a corneal equivalent. We also used co-cultures of DCs with fibroblasts (which are central to wound healing) to ascertain if DCs have a role in the excessive wound healing that follows hydrogel grafting, as previously reported by our team. To this end, the involvement of transforming growth factor (TGF)-ß, a hormone-like protein involved in the activation and recruitment of DCs in wound healing, was studied. We made the novel observation that DCs are, in fact, involved and they do this by secreting TGF-ß, which activates fibroblasts to produce the protein tenascin c, which is central to wound healing.

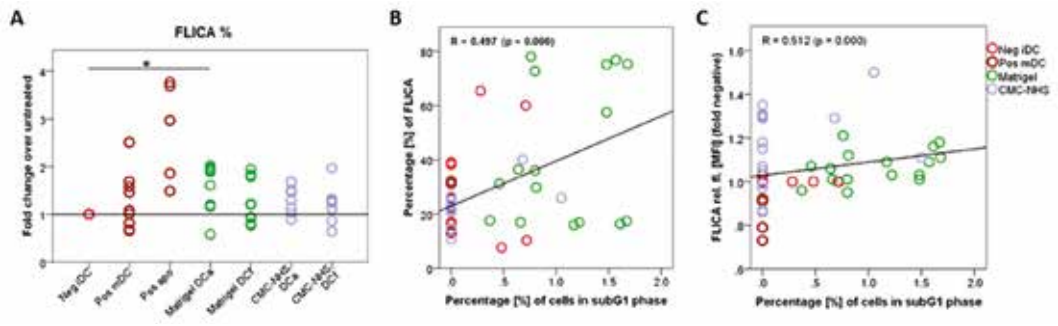
GRANT REPORT

(A) CLINICAL AND SCIENTIFIC SIGNIFICANCE OF ADVANCES MADE

Results summary – Study 1

To follow up on our previous evidence (Royal College of Surgeons of Edinburgh and Royal Blind grants) that particularly CMC-NHS (18% collagen) induced apoptosis in DCs (i.e., was tolerising), additional experiments including testing DCs on the natural matrix Matrigel and the hydrogels mentioned above were undertaken. To this end, fluorescent inhibitor of caspases (FLICA)-activated poly-caspases (Figure 1A) and 4',6-diamidino-2-phenylindole (DAPI) cell-cycle assays (Figure 1B, C) were done, together with experiments to quantify the markers of DC maturation CD86 and MHCII. Besides, to confirm qualitatively that the cells harvested were DCs, expression of the definitive DC transcription factor Zbtb46[1] (Figure 2A) was measured (together with the previously used surface markers CD11b and c). To ascertain if there were phenotypical differences between non-adherent cells (presumed to be DCs) in the culture and adherent cells (presumed to be macrophages)[2] both populations were analysed separately.

Nearly all the non-adherent cells were CD11b- and c-positive and expressed Zbtb46 simultaneously, confirming that the non-adherent cells contained most of the DCs in the culture (Figure 2A). We, therefore, worked with this population. Substantial apoptosis along with an increase in the number of cells in the subG1 cell-cycle phase were found (see statistical correlation analyses in Figure 1B, C). This was particularly true if cells were cultured on Matrigel, but also applied to CMC-NHS. Furthermore, apoptosis induction correlated with expression of the maturation markers CD86 and MHCII (Figure 2B–E).



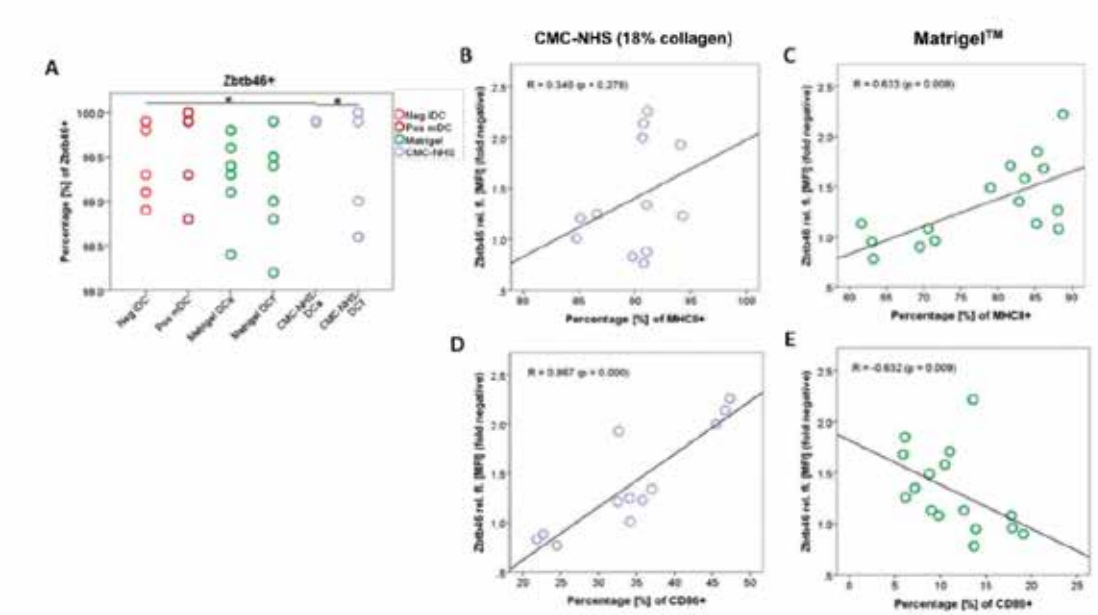
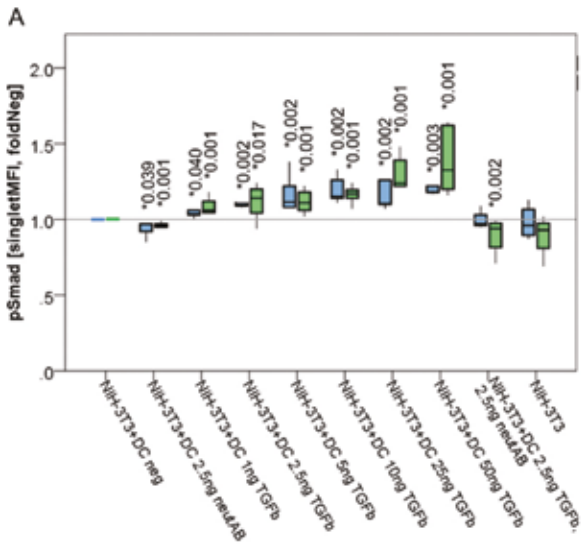


Figure 1 (top): Activated poly caspases (FLICA) expressed as percentages relative to the negative control (iDC – grey line; fold negative; (A)). * indicates significance on a 95% level of confidence (p≤0.05). For ease of reading, significant differences relative to the positive controls (mDC, Pos apo (apoptosis)) are not shown. DCa: DCs attached to the matrix (presumed to be macrophages); DCf: DCs floating over the matrix; CMC-NHS: RHCIII hydrogel 18% collagen. Correlations between activated poly caspases (FLICA) as percentages (B) and mean fluorescence intensity (MFI) (C), respectively, and the percentage of cells in the sub G1 cell-cycle phase (DAPI nucleophilic staining). Correlation graphs were generated using SPSS v24 applying the model of Spearman's rho for non-parametric data. Correlation coefficients (R) and p-values (p≤0.05 being significant (*)) are provided.

Figure 2 (above): Expression of the conventional DC-specific transcription factor Zbtb46 among different culture conditions (A). Percentages of Zbtb46 abundance were similar among those conditions tested and ranged between 98.6% and 99.9%. These findings confirm the immunological action of conventional DCs in the experiments. * indicates significance on a 95% level of confidence (p≤0.05). For ease of reading, significant differences relative to the positive control (mDC) are not shown. DCa: DC attached to the matrix (presumed to be macrophages); DCf: DCs floating over the matrix; CMC-NHS: RHCIII hydrogel 18% collagen. Correlations between the conventional DC-specific transcription factor Zbtb46 (as mean fluorescence intensity MFI) and the percentage of cells expressing increased levels of MHCII, upon culture on CMC-NHS 18% collagen (B) and Matrigel (C). Correlations with CD86 expression are shown for CMC-NHS 18% collagen (D) and Matrigel (E). Correlation graphs were generated using SPSS v24 applying the model of Spearman's rho for non-parametric data. Correlation coefficients (R) and p-values (p≤0.05 being significant (*)) are provided.

Results summary – Study 2 (ongoing Study 3 from previous report)

Data from our *in vivo* experiments in Study 2 of this project suggested that DCs might be involved in wound healing and generate a differential abundance of tenascin c by fibroblasts (involved in epithelial-to-mesenchymal transition)[3]. We followed up those data by co-culturing NIH-3T3 fibroblasts with DCs. DCs pre-treated with TGF-ß1 provoked phosphorylation of mothers against decapentaplegic homolog (Smad)2/3 in mouse fibroblasts (Figure 3) similar to the effect of treating fibroblasts with TGF-ß1 directly (Figure 4). These findings confirm earlier histological observations from confocal microscopy. When, in control experiments, fibroblasts were incubated directly with TGF-ß1, the results obtained were even more pronounced as compared with the co-culture condition, suggesting that TGF-ß was the specific inducer of tenascin c in fibroblasts and that tolerising DC generated TGF-ß to mediate this effect.



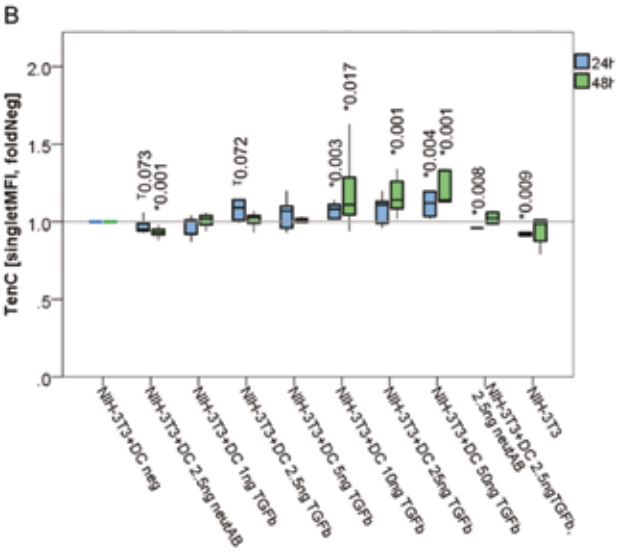
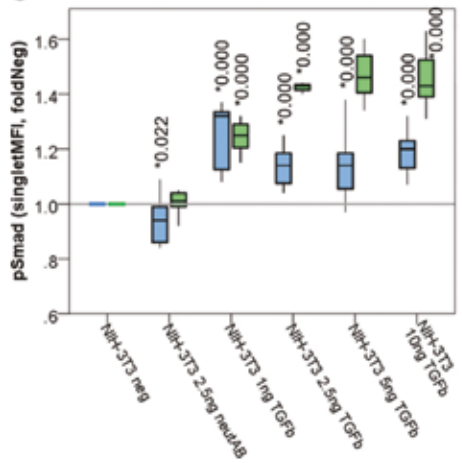


Figure 3: NIH-3T3 fibroblasts were co-cultured with DCs that had been (except for controls) pre-treated with TGF-ß1 over 24 h. After another 24 or 48 h, respectively, phosphorylation of Smad2/3 (A) and expression of tenascin c (B) was measured using flow cytometry. Both entities are indicative of a DC-mediated TGF-ß-dependent wound healing response in fibroblasts. Untreated cells and TGF-ß neutralisation antibody were used as control experiments. Group-wise comparison using the two independent samples Mann–Whitney *U*-test was based on skewed data distribution, and a 95% level of confidence. The number of cases/sample per group was 5–7. For ease of reading, only significant differences (*p-value) to negative control have been considered and are indicated in the boxplots. Graphs were generated using SPSS v24.

A



B

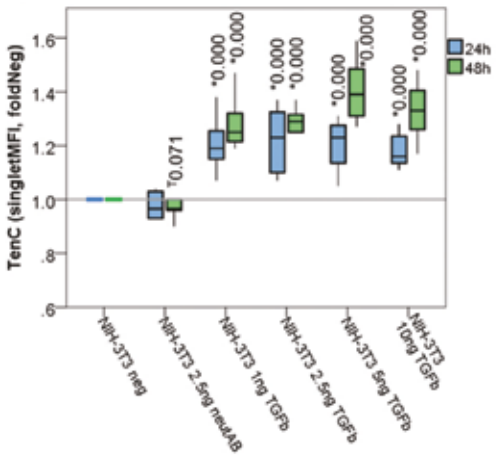


Figure 4: NIH-3T3 fibroblasts were cultured with and without TGF-ß1 over 24 and 48 h, respectively. Phosphorylation of Smad2/3 (A) and expression of tenascin c (B) were measured using flow cytometry. Both entities are indicative of a TGF-ß-dependent wound healing-type response in fibroblasts. Untreated cells and TGF-ß neutralisation antibody were used as control experiments. Group-wise comparison using the two-independent samples Mann–Whitney *U*-test were based on skewed data distribution, and a 95% level of confidence. The number of cases/samples was 5–7. For ease of reading, only significant differences (*p-value) to negative control have been considered and are indicated in boxplots. Graphs were generated using SPSS v24.

Conclusions

We investigated the effect of different collagen matrices on DC activation in the context of their use as corneal equivalents of corneal grafts. In previous experiments, it was difficult using current protocols for DC isolation to differentiate between DCs and macrophages. In the present study, we confirmed that the DC-specific transcription factor Zbtb46 was expressed in >90% of the DC population we used (CD11b+CD11c+ bone-marrow cells). We report that natural matrices such as corneal stroma and basement membrane-like matrices (Matrigel) modulate DC activation differentially compared with type I- and III-based collagen matrices. The fact that some matrices induced tolerance was revealed by further apoptosis and cell-cycle assays. These results were correlated significantly to markers of DC maturation.

We also found that tolerising DCs promoted wound-healing *via* production of TGF-ß as shown by phosphorylation of Smad2/3 and subsequent production of tenascin c. This was true when TGF-ß1 was administered directly to NIH-3T3 fibroblasts or if DCs that had been pre-treated with TGF-ß were co-cultured with fibroblasts. These findings emphasise a role for TGF-ß-mediated production of tenascin c in early wound healing, thereby promoting wound closure and potentially improving graft outcome based on a TGF-ß-based anti-inflammatory phenotype of DCs.

Scientific importance

Major advances were:

- Assessment and classification of different hydrogel matrices with varying chemistries with respect to induction of the innate immune response.
- Confirmation of a tolerising effect of some collagen gels on DCs.
- Demonstration of roles for tolerising DCs in mediating wound healing *in vitro*.

Clinical importance

This study will generate criteria for optimal design of hydrogels for supporting wound healing, tissue integration and sustained functionality for pre-clinical/clinical applications.

(B) PROBLEMS ENCOUNTERED AND STEPS TAKEN TO OVERCOME THEM

Scientific

TGF-ß1 neutralising antibody. Problem with titration. Resolved.

Logistical
Recurrent substantial delays in antibody deliveries. Resolved.

References

1. Satpathy AT, KC W, Albring JC, et al. Zbtb46 expression distinguishes classical dendritic cells and their committed progenitors from other immune lineages. *J Exp Med* 2012;209:1135–1152.
2. Inaba K, Inaba M, Romani N, et al. Generation of large numbers of dendritic cells from mouse bone marrow cultures supplemented with granulocyte/macrophage colony-stimulating factor. *J Exp Med* 1992;176:1693–1702.
3. Shankar SP, Griffith M, Forrester JV, et al. Dendritic cells and the extracellular matrix: a challenge for maintaining tolerance/homeostasis. *World J Immunol* 2015;5:113–130.

(C) COLLABORATIONS ESTABLISHED

- Professor May Griffith, Department of Clinical and Experimental Medicine, Linköping University, Linköping.
- Professor Keith Meek, School of Optometry and Vision Sciences, Cardiff University, Cardiff.

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

Publications

- Shankar SP, Griffith M, Forrester JV, et al. Dendritic cells and the extracellular matrix: a challenge for maintaining tolerance/homeostasis. *World J Immunol* 2015;5:113–130.
- Shankar SP, et al. Activation of dendritic cells by crosslinked collagen hydrogels (artificial corneas) varies with their composition [submitted].
- Shankar SP, et al. Dendritic cells and tenascin C in hydrogel corneal transplants [being written].

Funding

Royal College of Surgeons of Edinburgh, Major Project Ophthalmology Grant, 2016–2017 (£49,971).

(E) ACKNOWLEDGEMENTS

We acknowledge the Royal College of Surgeons of Edinburgh and Royal Blind for funding received during 2014–2017. We thank the confocal microscopy, flow cytometry, qPCR facilities and Medical Research Facility staff at the University of Aberdeen for technical assistance.

Ophthalmology Grant Report

Evaluation of antimicrobial peptide synergism against ocular surface pathogens

Professor Harminder S Dua, Dr Imran Mohammed and Mrs Dalia G Said
Academic Section of Ophthalmology, Division of Clinical Neuroscience, Queen's Medical Centre, Nottingham
July 2016 to December 2017

LAY SUMMARY

Microbial keratitis is a common cause of preventable unilateral blindness worldwide. Resistance to antibiotics is an emerging global problem and alternative therapies have been sought. Antimicrobial peptides (AMPs) are broad-spectrum naturally occurring antibiotic analogues produced by mammalian cells in response to inflammation or infection. Previously, we demonstrated that expression of human beta-defensin (HBD)9 is reduced during microbial infections, unlike that of other AMPs such as HBD2, HBD3 and the antimicrobial peptide LL37, which is increased.

We showed that a linear HBD9 peptide (14 amino acids in length) could kill a range of bacteria (methicillin-resistant *Staphylococcus aureus* (MRSA), methicillin-sensitive *Staphylococcus aureus* (MSSA), two strains of *Pseudomonas aeruginosa* (PA01L and PA-OS) and methicillin-resistant *Staphylococcus epidermidis* (MRSE)) at <25 mM *via* membrane disruption. HBD9 peptide in combination with LL-37 could kill MRSA and PA-OS at very low concentrations (<6.25 mM). We also identified expression of miRNA-155 and miRNA-146a to be upregulated in corneal epithelial cells treated with PA exoproducts. However, their role in downregulation of HBD9 expression merits further characterisation.

Our study has provided an essential platform to pursue the therapeutic development of HBD9 and LL-37 combination peptides for treatment of drug-resistant pathogens.

GRANT REPORT

(A) CLINICAL AND SCIENTIFIC SIGNIFICANCE OF ADVANCES MADE

According to European Committee on Antimicrobial Susceptibility Testing (EUCAST) guidelines, we evaluated antibacterial activities (minimum inhibition concentration (MIC) using the broth-microdilution method) of recombinant HBD2 and HBD3 proteins as well as LL-37 and HBD9 full-length peptides against MRSA, MSSA, PA01L, PA-OS and MRSE. MRSA and PA-OS were ocular surface-isolated clinical strains and MRSE was from a patient with a urinary-tract infection (collected from Professor Roger Bayston, Department of Clinical Microbiology, University of Nottingham). MSSA and PA01L were laboratory strains procured from Drs. Ewan Murray and Stephan Heeb (Department of Molecular Microbiology, University of Nottingham).

Organism		MIC (µg/mL)				MIC (µM)		
		HBD2	***HBD3***	***LL-37***	***HBD9***	***HBD9-8***	***HBD9-14***	***HBD9-21***
Gram-positive bacteria	***MRSA***	*N.A.*	100	25	*N.A.*	*N.A.*	25	*N.A.*
	MRSE	*N.A.*	25	12.5	62.5	*N.A.*	12.5	*N.A.*
	MSSA (SH1000)	*N.A.*	50	12.5	*N.A.*	*N.A.*	25	*N.A.*
Gram-negative bacteria	***PA-OS***	*N.A.*	100	50	*N.A.*	*N.A.*	25	*N.A.*
	PA01L	100	50	12.5	*N.A.*	*N.A.*	25	*N.A.*

N.A. = No activity

Table 1: MICs of AMPs according to EUCAST guidelines.

As expected, recombinant (r)HBD3 and LL-37 demonstrated microbicidal activity against all tested bacteria (Table 1). rHBD2 and HBD9 full-length peptide were efficacious against PA01L and MRSE, respectively. To understand the role of various cysteines and hydrophobic C-terminal sequences, we also generated three small peptides of HBD9 (8, 14 and 21 amino acids in length). Interestingly, HBD9 peptide (length = 14 amino acids) demonstrated potent bactericidal activity (Table 1). In the presence of physiologic salt, HBD9–14 activity was reduced slightly and required 2× MIC to kill PA-OS and MRSA (Table 2). We also demonstrated that combination of HBD9 and LL-37 peptides had an additive killing effect against MRSA (Table 3).

MIC (µM)	0mM NaCl	150 mM NaCl
MRSA	25	50
PA-OS	12.5	25

Table 2: MICs of HBD9-14 peptide in the presence or absence of physiologic salt content.

[a] FICI	LL-37
HBD9-14	1

Table 3: Fractional Inhibition Concentration Index (FICI) calculated for two peptides in combination by the checker-board method.

[a]FICI = (MIC A_{comb} /MIC A_{alone}) + (MIC B_{comb} /MIC B_{alone})
FICI < 0.5 represents synergism
FICI between 0.5 and 2 represents an additive effect
FICI < 2 represents an inhibitory effect

Furthermore, we elucidated the membrane-disruption properties of HBD9 peptide against MRSA using the SYTOX™ Green permeability assay. The latter was carried out in two conditions: (a) 5% tryptic soy broth (TSB) at different pH (6.8, 7.2 and 7.6); b) 5% TSB with or without 150 mM of NaCl (physiologic concentration of salt in tears). Relative fluorescence units (RFUs) were calculated compared with a positive-control peptide, melittin (a strong membrane disruptor secreted by *Apis apidae*). At 1× MIC, HBD9–14 killed bacteria by increasing membrane permeability (80% activity compared with melittin) in the pH range 6.8–7.6 in tears (Figure 1).

However, in the presence of 150 mM of NaCl the activity of HBD9–14 was reduced (Figure 2). Further experiments at 2× MIC should validate the findings in Table 2 and Figure 2.

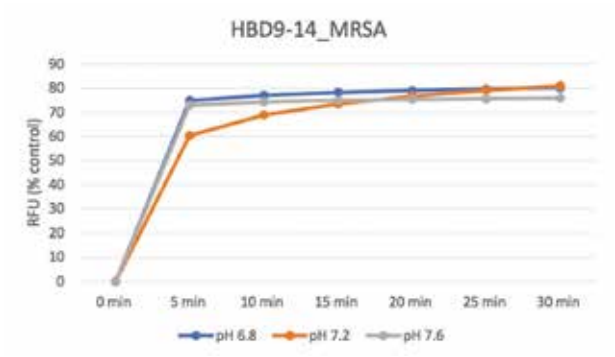


Figure 1: SYTOX Green permeability assay using 1× MIC of HBD9 small peptide against MRSA at different pH.

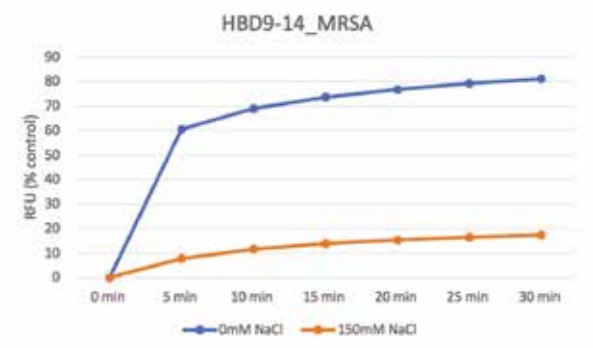


Figure 2: SYTOX Green permeability assay using 1× MIC of HBD9 small peptide against MRSA at physiologic salt concentration.

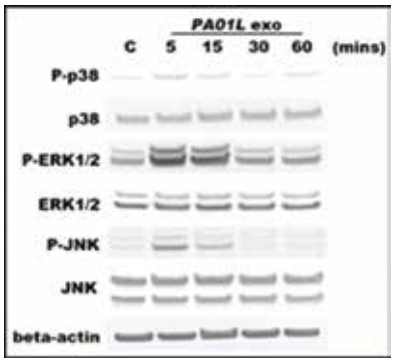


Figure 3: Immuno analyses of MAPK and NF-kB signalling pathways in response to PA01-L exoproduct.

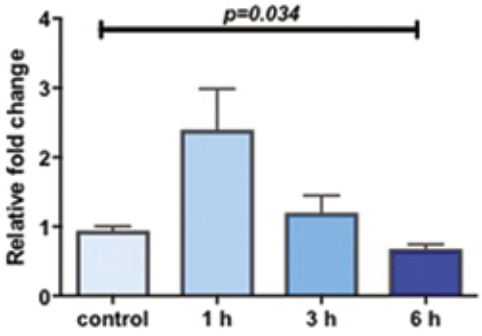


Figure 4: Analyses of HBD9 expression in response to PA-01L exoproduct.

Numerous studies have provided substantial insight into host–pathogen interactions with live or heat-killed bacterial suspensions, or synthetic ligands. However, less is known about the impact of products synthesised and secreted by the bacteria on host cells. To elucidate the mechanism by which microbial proteins might reduce HBD9 in the corneal epithelium, we studied the effect of *P. aeruginosa* (PA-01L strain) exoproducts on activation of mitogen-activated protein kinase (MAPK) signalling pathways. We noted immediate activation of all MAPK signalling molecules in response to PA-01L exoproduct (Figure 3). Similar to our previous studies, PA-01L exoproduct also reduced expression of HBD9 in corneal epithelial cells (Figure 4). Of the miRNAs tested, expression of only miR-155 and miR-146a were increased in a time-dependent fashion (Figure 5). Intriguingly, the pattern of reduction of expression of HBD9 matched the miRNA induction in response to PA-01L exoproduct, suggesting a potential role of miR-155 and miR-146a in suppression of HBD9 levels during PA-01L-induced keratitis.

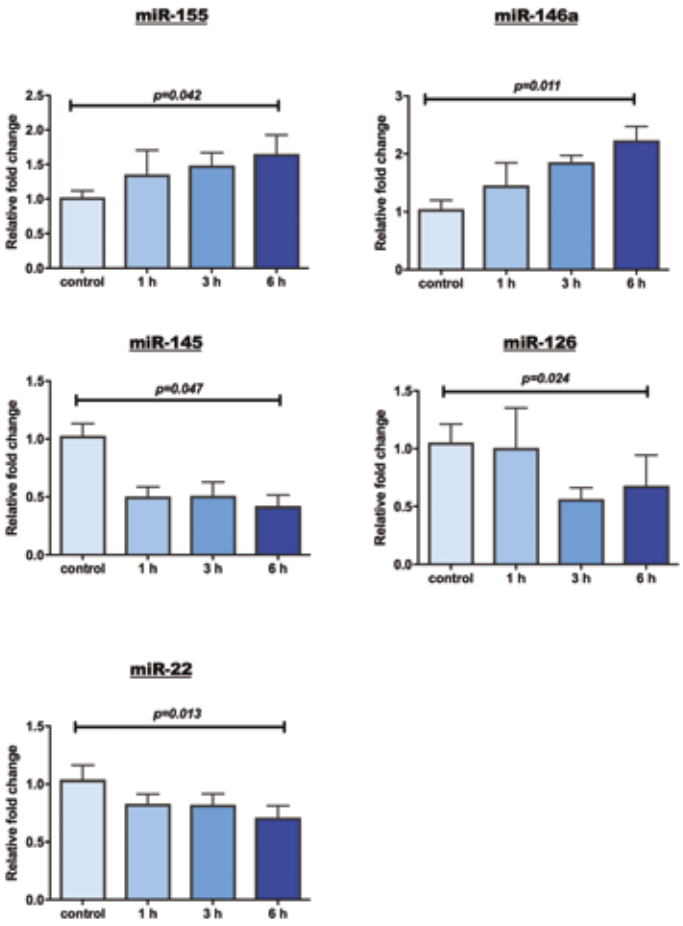


Figure 5: Analyses of Toll-like receptor regulatory miRNAs in corneal epithelial cells challenged with PA-01L exoproduct.

Further studies are warranted to dissect the role of miRNAs in regulation of HBD9 expression on the corneal epithelium, which could provide insight into the pathogenesis of microbial keratitis. Moreover, studying the killing mechanism of HBD9 could enable development of therapies for sight-threatening infectious conditions.

(B) PROBLEMS ENCOUNTERED AND STEPS TAKEN TO OVERCOME THEM

Our initial aim was to generate rHBD9 in collaboration with Professor Mark Searle (Department of Chemistry, University of Nottingham). Due to unusually long hydrophobic C-terminal sequences, HBD9 started to precipitate. Hence, we constructed various lengths of HBD9 linear peptides commercially. Similar to rHBD9, the full-length peptide of HBD9 (65 amino acids) precipitated upon circularisation. Of three small peptides we constructed, a peptide of length 14 amino acids showed very strong antimicrobial properties against a range of Gram-negative and Gram-positive bacteria.

(C) COLLABORATIONS ESTABLISHED

The collaboration with Professor Mark Searle has enabled generation of smaller HBD9 peptides. In the future we aim to jointly apply for major funding from the Biotechnology and Biological Sciences Research Council (BBSRC) to study the structure of the potent HBD9 small peptide using circular dichroism and nuclear magnetic resonance (NMR) to understand the interaction between bacterial proteins with HBD9.

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

- Mohammed I, Said DG, Dua HS. Human antimicrobial peptides in ocular surface defense. *Prog Retin Eye Res* 2017;61:1–22.

Intellectual property will be filed with help from the Research & Innovation Unit of the University of Nottingham to protect the small peptide of HBD9 and its application for treatment of drug-resistant pathogens. Once intellectual property has been filed, we will publish our study results in an open-access journal.

We will aim to apply for a MRC Confidence in Concept grant and/or Fight for Sight major grant to test the *in vivo* efficacy of HBD9 small peptide against MRSA and PA-01L.

(E) ACKNOWLEDGEMENTS

We acknowledge financial support from the Royal College of Surgeons of Edinburgh and Royal Blind.

Ophthalmology Grant Report

Optimising gene therapy treatments to improve patient safety

Professor Robert E MacLaren
Nuffield Laboratory of Ophthalmology, University of Oxford
1 October 2016 to 30 September 2017

LAY SUMMARY

The retina is the light-sensitive layer that lines the back of the eye, and which allows us to see. Most incurable forms of blindness are due to genetic diseases, which are caused by faulty genes in the DNA of retinal cells. These faulty genes eventually lead to the dysfunction and death of affected cells.

Gene therapy is a new method which involves putting normal copies of the faulty gene back into retinal cells to help them to function normally. Gene therapy uses AAV vectors to carry normal genes into retinal cells.

Bacteria are used in the initial manufacturing step for the production of AAV vectors. In this initial phase, there is a risk that bacterial DNA may be incorporated mistakenly into the vector particles instead of the healthy copies of the gene in question. The presence of bacterial DNA can lead to inflammation after the suspension of AAV vectors is injected into the retina. We aimed to reduce the amount of bacterial DNA by modifications to the production method of AAV vectors. This strategy could improve patient safety for people undergoing gene therapy with AAV vectors in clinical trials.

GRANT REPORT

(A) CLINICAL AND SCIENTIFIC SIGNIFICANCE OF ADVANCES MADE

We remain extremely grateful to the Royal College of Surgeons of Edinburgh for its long-standing support. Such support has enabled us to pursue a highly successful translational research programme developing novel gene therapies for the treatment of previously incurable forms of blindness. This success led in 2014, through the support of the Wellcome Trust, to the establishment of Nightstar Therapeutics, a company specialising in retinal gene therapy spun out of our research programme at the University of Oxford. Building on the AAV technology developed in previous research projects funded by the Royal College of Surgeons of Edinburgh, Nightstar Therapeutics launched in early 2017 the world's first clinical trial of a gene therapy for X-linked RP at the Oxford Eye Hospital. In addition, Nightstar Therapeutics will shortly be commencing an international phase-3 clinical trial of our gene therapy for choroideremia. 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.

Background to the current project

The wild-type AAV genome comprised ≈4.7 kb of single-stranded DNA. Under optimal packaging conditions, AAV vectors can be designed containing ≤5.1 kb of DNA. Recombinant AAV vectors are constructed such that the therapeutic gene in question ('transgene') is flanked by palindromic sequences from the wild-type AAV genome known as inverted terminal repeats (ITRs). The latter are critically important in vector production because they guide internalisation of the single-stranded DNA into the AAV capsid and facilitate assembly of the capsid proteins around the transgene. ITRs also assist in stabilisation of the AAV transgene after transduction in host cells and facilitate the long-term stability of the AAV transgene by linking together to form circular double-stranded DNA structures ('concatemers').

The initial manufacturing step for the production of AAV vectors involves inserting the AAV expression cassette within a bacterial plasmid and cloning the plasmids in bacterial cultures. The plasmids are then removed from the bacteria, purified, and introduced into human cells, in which production of the recombinant AAV vectors takes place. The packaging of transgenes

Construct	Serotype	Backbone size	Transgene size	Stuffer	Resistance
#1	AAV8 Y733F	2277	4800		AmpR
#2	AAV8 Y733F	2277	4500		AmpR
#3	AAV8 Y733F	2277	4300		AmpR
#4	AAV2	3372	3970		AmpR
#5	AAV2	3372	3800		AmpR
#6	AAV2	3377	3370		AmpR
#7	AAV2	3377	3100		AmpR
#8	AAV2	3374	2700		AmpR
#9	AAV2	3374	2100		AmpR
#10	AAV2	5487	3800	Yes	KanR
#11	AAV2	5516	3600	Yes	KanR
#12	AAV2	5551	2500	Yes	KanR

Table 1: AAV preparations used in qPCR

into AAV capsids is considered to be directional, with the ITRs guiding this step. However, packaging of DNA in the wrong direction can occur, such that the bacterial backbone DNA is packaged into the AAV capsid instead of the transgene. Such 'reverse packaging' may account for 5–8% of the total dose of the AAV vector administered. Bacterial DNA can be recognised by the intracellular immune system of the host as being abnormal and may, in theory, drive a late inflammatory response that might otherwise hinder successful AAV transduction in a particular cell.

Relatively high doses of AAV vector are, in general, administered in retinal gene therapy to maintain adequate expression of wild-type genes. These high viral doses further increase the risk of inflammation. We wished to optimise vector production so as to reduce the risks of inflammation and thereby improve patient safety and treatment efficacy. Specifically, we aimed to assess the proportion of bacterial plasmid DNA packaged into a given preparation of AAV vectors, and investigate if this proportion could be reduced by modification of the bacterial plasmid containing the AAV expression cassette.

Specific objectives of our project

- Calculate the proportion of contaminating bacterial DNA in the vector preparation.
- Underake AAV titration by qPCR using primers to the transgene region of the AAV (targeting the polyA region).
- Carry out AAV titration by qPCR using primers to the antibiotic resistance gene located in the backbone of the AAV plasmid (ampicillin (AmpR) or kanamycin (KanR)).
- Calculate the percentage of reverse packaging (ratio of the titre obtained targeting the resistance gene to the titre obtained targeting the AAV transgene).
- Document evidence of bacterial DNA transduction in the retina *in vivo*.
- Assess the effects of a modified plasmid in reducing bacterial DNA contamination.

Results

The data obtained from the project so far highlight the importance of our research. Various AAV preparations generated from plasmids carrying different transgene and backbone structures were assessed for transgene and antibiotic resistance gene titre (Table 1). Primers were designed to target the polyA region within the transgene and the antibiotic resistance gene in the original plasmid backbone structure (AmpR or KanR).

The three constructs that contained KanR also contained a stuffer sequence in the backbone structure of 2,500 bp.

The 12 AAV preparations were processed for qPCR (Dnase-treated AAV preparations were run in two independent assays with three replicates each). The final titrations representing genome copies of packaged DNA per millilitre of AAV are presented in Figure 1. The proportion of antibiotic resistance gene detected in each AAV preparation is depicted in Figure 2.

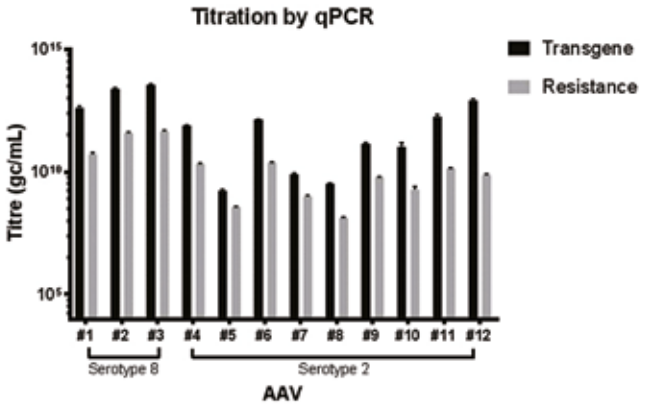


Figure 1: Titration of AAV samples by qPCR. Titres obtained using primers targeting the transgene region are shown in black. Grey bars represent titres obtained using primers targeting the antibiotic resistance gene in the AAV plasmid backbone. Values are mean of 6 replicates ± SEM.

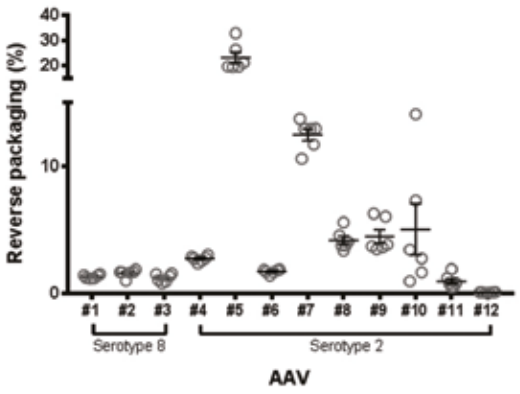


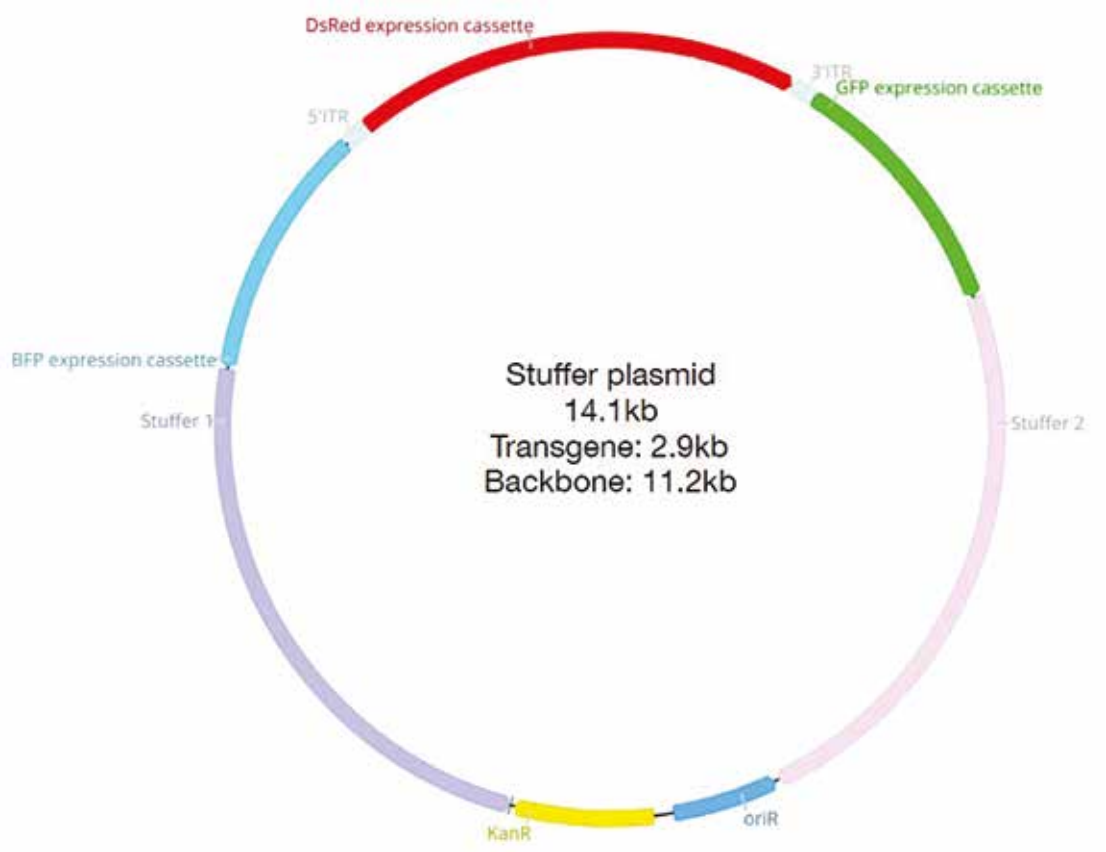
Figure 2: Percentage of antibiotic resistance genes detected in AAV preparations. The relative contribution of the resistance gene titre to the overall AAV titre is shown in circles (6 replicates for each AAV). Error bars represent the mean ± SEM.

These data enabled us to draw some conclusions and highlighted areas of interest regarding reverse packaging:

- The percentage of reverse packaging in AAV preparations is variable and some of this variation is user-dependent (four users made AAV preparations 1–12 on different occasions).
- The ITR sequence may contribute to the variability of reverse packaging (AAV #7, #8 and #9 *versus* all others).
- A stuffer sequence in AAV2 preparations appears to reduce the percentage of KanR packaging in 2 out of 3 AAV tested (#11 and #12).

Our data confirmed that reverse packaging is a potential problem in AAV production. A systematic approach must be employed to determine the benefits of a stuffer sequence in reducing bacterial gene packaging. To this end, a new study has been designed to investigate the prevalence of reverse packaging from each ITR, the potential influence of backbone size on this prevalence and, subsequently, the level of antibiotic resistance packaging. A specialised plasmid, p14, has been constructed (Figure 3), key features of which include three expression cassettes for ubiquitous expression of red, blue and green fluorescent proteins. These will act as markers of expression for transgene and backbone packaging in AAV preparations. Construct p14 also contains two regions of a stuffer sequence that can be removed independently to generate plasmid variants p10A, p10B and p7, all containing identical transgenes but with varying backbone structures (Figures 3A, B and C, respectively). Plasmid p14 has been cloned and tested *in vitro* for expression of these three fluorescent proteins (Figure 4). Generation of plasmid variants p10A, p10B and p7 is ongoing. The intention is to use four plasmid constructs to generate AAV preparations that can be assessed for the qPCR titre of transgene and backbone elements to identify packaging ratios. Additional *in vitro* assessment of expression of fluorescent proteins will be undertaken following treatment with each AAV preparation.

The ultimate aim is to ascertain if inclusion of a stuffer sequence in the backbone of an AAV gene therapy construct reduces the levels of bacterial gene present in the resulting AAV preparation. Achieving this aim could reduce adverse immune reactions to bacterial DNA, thereby making gene therapy safer for patients.



A) p10A B) p10B C) p7

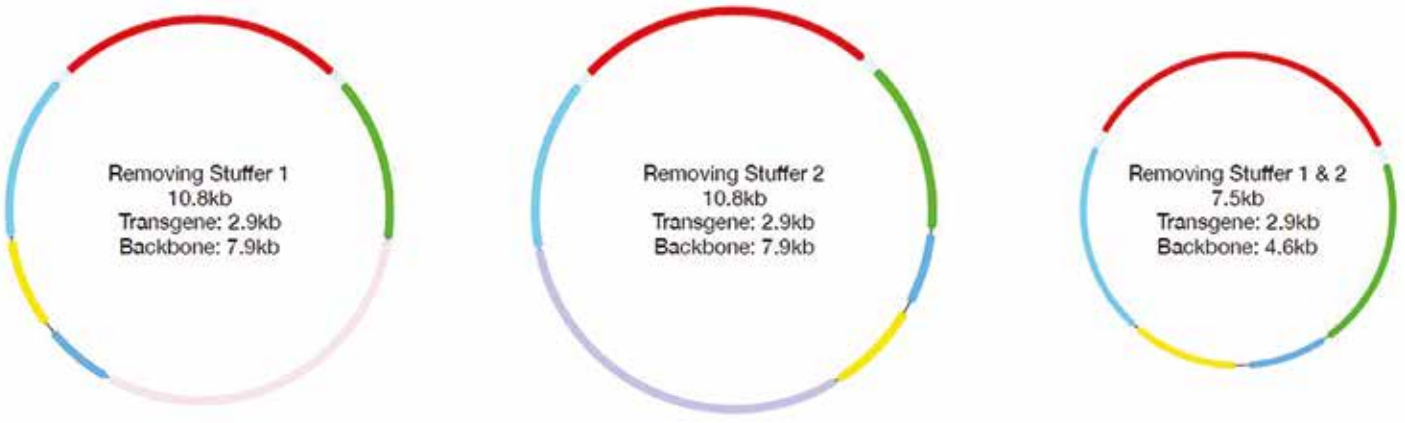


Figure 3: Design of the plasmids being used in ongoing investigations. The plasmid p14 (upper construct) contains a typical transgene structure comprising a ubiquitous promoter (CAG) driving expression of DsRed (red fluorescent protein) between AAV2 ITRs. On either side of the ITRs are small-expression cassettes for generation of mTagBFP (blue fluorescent protein) or GFP (green fluorescent protein) with the kanamycin resistance gene (KanR) lying at the halfway point in the plasmid backbone. Three further plasmids can be generated by excision of either stuffer sequences 1, p10A (3A) and 2, p10B (3B) or both, p7 (3C).

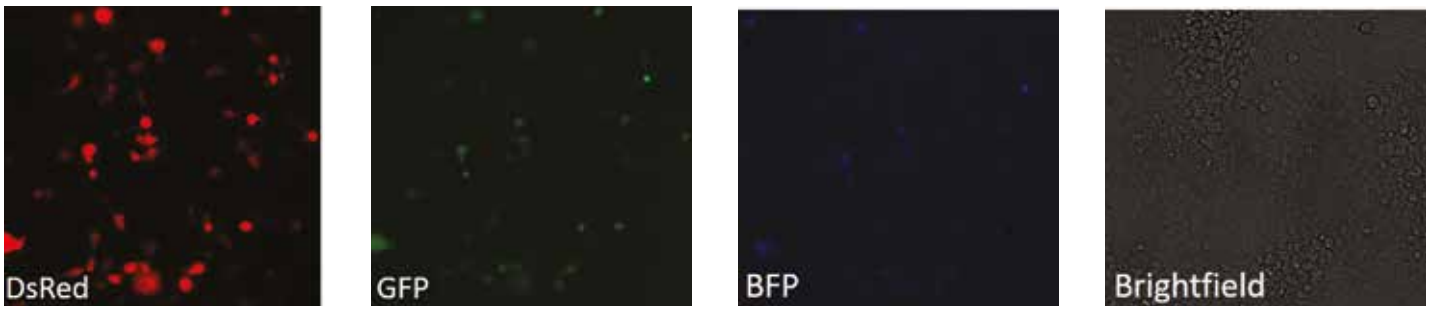


Figure 4: *In vitro* expression of three fluorescent proteins following transfection of 293 cells with plasmid DNA. Upon arrival in our laboratory, the synthesised plasmid was cloned with 1 µg used for transfection of mammalian cells (293 cell line). Expression of the reporter proteins was confirmed 4 days post-transfection using specific channels and filters of a fluorescence microscope. Scale bar = 200 µm.

(B) PROBLEMS ENCOUNTERED AND STEPS TAKEN TO OVERCOME THEM

One of the most important steps before qPCR is to ensure primer pairs can detect target sequence levels reliably. Primers for the polyA region in the transgene had been optimised and tested previously. However, primers to detect the antibiotic resistance genes were new and required optimisation. Appropriate primer sets were designed and tested, with the most efficient selected for qPCR-based titre assessment of AAV preparations.

(C) COLLABORATIONS ESTABLISHED

A joint research programme has been set up between the MacLaren research team and Gyroscope Therapeutics. The latter is a newly established biopharmaceutical company that aims to develop gene therapies for the treatment of eye diseases linked to an unbalanced complement system. Over-activation of the complement system has been associated with development of dry age-related macular degeneration (AMD). Hence, gene therapies that can restore the equilibrium of the complement system may slow progression of dry AMD significantly and prevent vision loss.

Dry AMD (which develops if the cells of the macula become damaged by a build-up of deposits called 'drusen') is the most common type of AMD, accounting for ≈9 out of 10 cases. It presents as a progressive and debilitating loss of vision in the centre of the visual field (macula). As the disease progresses to the atrophic form (which is characterised by loss of the retinal pigment epithelium, leading to degeneration of nearby photoreceptors), the corresponding loss of central vision prevents recognition of faces or the ability to drive motorised vehicles, read, or perform other activities of daily life.

AMD affects >600,000 people in the UK and is the leading cause of vision loss. AMD prevalence increases significantly with age, with >10% of the population >70 years of age showing signs of AMD. By 2020, it is predicted that ≈700,000 people in the UK will have late-stage AMD.

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

Prizes

Dr Alun Barnard, a Senior Postdoctoral Research Fellow who supported this and preceding Major Project Grants in Ophthalmology, was awarded the Sherrington Poster Prize at the 8th Oxford Neuroscience Symposium in March 2017. His poster was entitled 'Investigating cell fusion between the donor and host retina after photoreceptor precursor transplantation'. This work was based on previous research projects funded by the Royal College of Surgeons of Edinburgh and Royal Blind entitled 'Development of photoreceptor transplantation in the totally degenerate retina' and 'Development of cone photoreceptor transplantation', which were undertaken from October 2013 to September 2015.

Publications

The experimental results of previous research projects funded by the Royal College of Surgeons of Edinburgh, 'Preclinical testing of a new gene therapy vector for Stargardt disease' and 'Optimisation of ABCA4 gene expression as a treatment for Stargardt disease', undertaken from October 2011 to September 2013, have been published in the *Journal of Genetic Syndromes and Gene Therapy*:

- McClements ME, Charbel Issa P, Blouin V, et al. A fragmented adeno-associated viral dual vector strategy for treatment of diseases caused by mutations in large genes leads to expression of hybrid transcripts. *J Genet Syndr Gene Ther* 2016;7.pii:311.

The experimental results of previous research projects funded by the Royal College of Surgeons of Edinburgh, 'Development of photoreceptor transplantation in the totally degenerate retina' and 'Development of cone photoreceptor transplantation', undertaken from October 2013 to September 2015, have been published in *Nature Communications*:

- Singh MS, Balmer J, Barnard AR, et al. Transplanted photoreceptor precursors transfer proteins to host photoreceptors by a mechanism of cytoplasmic fusion. *Nat Commun* 2016;7:13537.

Several other scientific articles, generated from ongoing research projects based on preliminary work supported by the Royal College of Surgeons of Edinburgh, have been published during 2017. These publications, which have all acknowledged the original support of the Royal College of Surgeons of Edinburgh, are:

- De Silva SR, Charbel Issa P, Singh MS, et al. Single residue AAV capsid mutation improves transduction of photoreceptors in the Abca4−/− mouse and bipolar cells in the rd1 mouse and human retina *ex vivo*. *Gene Ther* 2016;23:767–774.

- Simunovic MP, Jolly JK, Xue K, et al. The spectrum of CHM gene mutations in choroideremia and their relationship to clinical phenotype. *Invest Ophthalmol Vis Sci* 2016;57:6033–6039.

- Patrício MI, Barnard AR, Orlans HO, et al. Inclusion of the woodchuck hepatitis virus posttranscriptional regulatory element enhances AAV2-driven transduction of mouse and human retina. *Mol Ther Nucleic Acids* 2017;6:198–208.

- Han RC, Jolly JK, Xue K, et al. Effects of pupil dilation on MAIA microperimetry. *Clin Exp Ophthalmol* 2017;45:489–495.

- Fischer MD, McClements ME, Martinez-Fernandez de la Camara C, et al. Codon-optimized rpgr improves stability and efficacy of AAV8 gene therapy in two mouse models of x-linked retinitis pigmentosa. *Mol Ther* 2017;25:1854–1865.

- Salvetti AP, Patrício MI, Barnard AR, et al. Impact of vital dyes on cell viability and transduction efficiency of AAV vectors used in retinal gene therapy surgery: an *in vitro* and *in vivo* analysis. *Transl Vis Sci Technol* 2017;6:4.

(E) ACKNOWLEDGEMENTS

We thank the Royal College of Surgeons of Edinburgh and Royal Blind for their generous sponsorship of this and preceding Major Project Grants in Ophthalmology, which have underpinned the 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

Are we carrying out cataract surgery too early? Evaluation of patient-reported outcomes

Umiya Agraval
ST3, Tennents Institute of Ophthalmology, Gartnavel General Hospital, Glasgow
December 2016 to May 2018

LAY SUMMARY

Cataract surgery is the most common elective surgical procedure carried out in the UK. It can improve not only visual acuity, but also generic health and vision-specific qualify of life (QoL) measures dramatically. In recent years there has been significant lowering of the threshold for surgical intervention for cataracts. Procedures are being carried out at an earlier stage, sometimes before the general wellbeing of the person is seriously affected.

Assessment of the perspectives of patients by measuring patient-reported outcomes (PROs) gives a more realistic indication of the benefit of cataract surgery. Based on our clinical observations, we expect that early extraction of cataracts may not lead to significant improvements in PROs in a substantial proportion of patients. We propose to measure PROs in patients with mild cataracts to ascertain if early surgery is beneficial. Our study is ongoing.

GRANT REPORT

(A) CLINICAL AND SCIENTIFIC SIGNIFICANCE OF ADVANCES MADE

Having an accurate perspective from patients of the benefits of cataract surgery is an important factor to consider, and may be different from the surgeons' perspective. Patients' perspectives should guide the timing of surgical intervention and help determine the scale of future service provision. The concept of PROs is gaining support rapidly in ophthalmology. Development of tools to measure PROs aimed at measuring general health status, vision function, and visual function-related QoL is developing rapidly. Our work may answer important questions on the efficacy of early cataract surgery.

We anticipate that the results of this study will show that intervention for early cataracts in a proportion of cases is not effective in improving PROs. We believe there is urgent need to re-think the way we assess patients for cataract surgery. If our hypothesis proves to be correct, the data will support better selection of patients and avoidance of unnecessary surgery.

Results

We have recruited 32 patients (22 females, 10 males; mean age, 70.68 years; age range, 52–83 years). Fifteen patients had first-eye and 17 patients had second-eye cataract procedures.

Preoperative visual acuity (VA) was 6/9 in 44%, 6/7.5 in 6%, 6/6 in 38%, and 6/5 in 12% of patients having cataract procedures. The distribution of questionnaire scores preoperatively is shown in Figure 1. For the visual function (VF)-14 questionnaire, the higher the score the higher was the visual function. For the impact of visual impairment (IVI) questionnaire, the lower the score the better was the vision-related QoL. The mean VF-14 questionnaire score preoperatively was 75.62 (range, 52–100) and the mean IVI questionnaire score was 16.22 (range, 1–56).

We have 4 months of data on 18 patients (Figure 2) and have undertaken statistical analyses on these data. Pearson's r was calculated to assess the correlation between age and QoL questionnaire score. There was a weak positive correlation between age and VF-14 score ($r = 0.24$) and weak negative correlation between age and IVI score ($r = -0.23$). These data sugges a slight trend towards better QoL with increasing age.

The Student's *t*-test was used to assess differences in questionnaire scores preoperatively to postoperatively (Figure 3, 4). There was a significant improvement in VF-14 (mean difference, 14.43, $t<0.001$) and IVI (mean difference, −8.95, $t = 0.03$) preoperatively and 4 months postoperatively (Figure 5, 6).

The mean QoL scores for patients undergoing first-eye

surgery *versus* second-eye surgery were also compared. Overall, QoL appeared better for second-eye procedures, approaching significance for VF-14 (mean difference, 8.45, t=0.07) but significant at the 1% level for IVI (mean difference, –10.87, t=0.01).

(B) PROBLEMS ENCOUNTERED AND STEPS TAKEN TO OVERCOME THEM

Our calculations on the statistical power of the study suggest that we must recruit 200 patients for the study. However, we have only recruited 32 individuals so far.

We have had difficulty identifying patients with no comorbidities that fit the inclusion criteria, especially at the NHS Greater Glagow and Clyde Trust. We have added a further site, NHS Golden Jubilee National Hospital, which has high cataract surgery outflow. This has increased our recruitment numbers. We have extended the study period to accommodate higher recruitment numbers.

(C) COLLABORATIONS ESTABLISHED

- Dr Patrick Kearns, Lead Consultant Ophthalmologist – Collaborator at NHS Golden Jubilee National Hospital.
- Eilidh Farquhar, Research Optometrist – Employed using the research grant to work 1 day a week for the study. Mainly involved in the recruitment process, assessment of cataract grading for recruited patients, and helping patients complete the questionnaires.

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

Currently recruiting patients. We hope the data we collate can be used to establish further funding to expand the study to the national level.

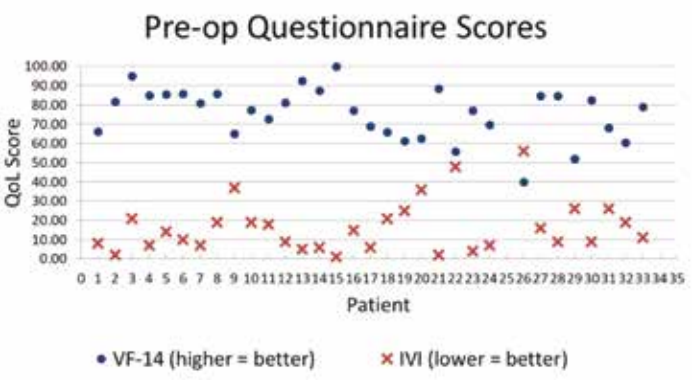


Figure 1: Questionnaire scores for VF14 and IVI preoperatively.

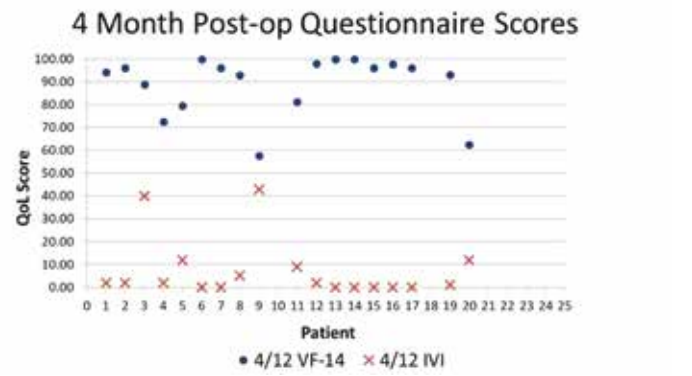


Figure 2: Questionnaire scores for VF14 and IVI 4 months after cataract surgery.

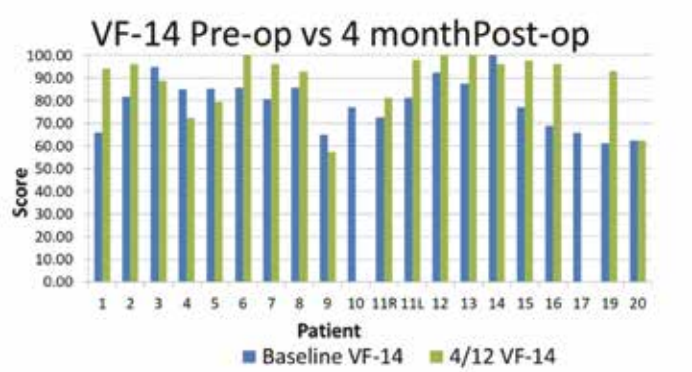


Figure 3: Comparison of the VF-14 questionnaire score before and 4 months after cataract surgery.

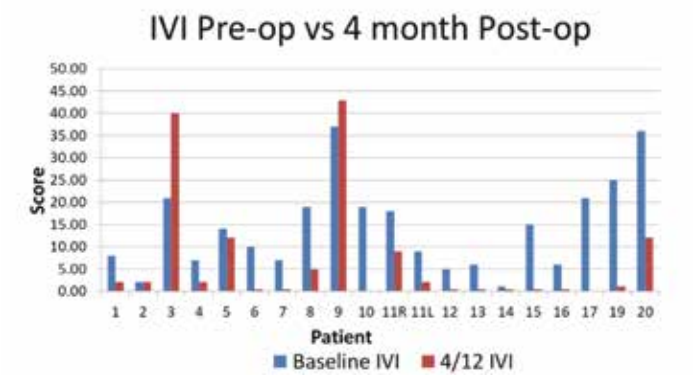


Figure 4: Comparison of IVI questionnaire score before and 4 months after cataract surgery.

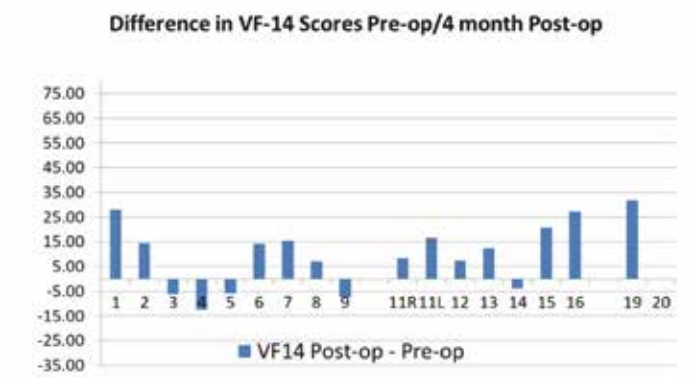


Figure 5: Difference in VF-14 questionnaire score before and 4 months after cataract surgery.

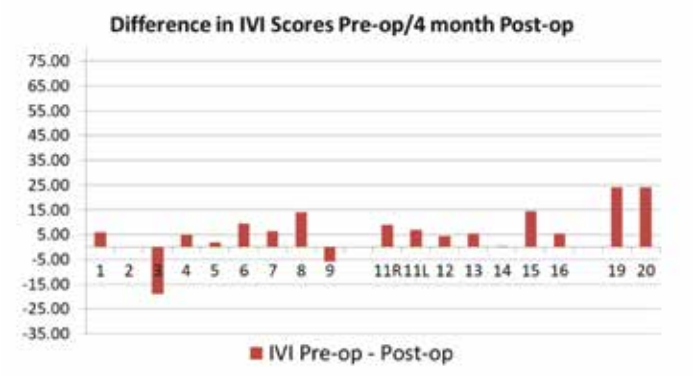


Figure 6: Difference in the IVI questionnaire score before and 4 months after cataract surgery.

Ophthalmology Grant Report

Pre-Descemet's endothelial keratoplasty: understanding the science and developing the surgery

Professor Harminder S Dua
Academic Section of Ophthalmology, Division of Clinical Neuroscience, University of Nottingham
May 2016 to April 2017

LAY SUMMARY

Transplantation of the cornea (the clear window of the eye) involves replacement of only the diseased layers in the front (deep anterior lamellar keratoplasty (DALK)) or back of the eye (endothelial keratoplasty (EK)). One form of EK is pre-Descemet's endothelial keratoplasty (PDEK), wherein the innermost layer of the cornea together with the recently discovered pre-Descemet's layer (PDL) is transplanted. The advantages of PDEK are that, like other procedures, it gives good visual results and reduces the risk of rejection but, in addition, it is easier to carry out and exclusively permits tissue from very young donors (with a larger number of cells) to be used.

We designed, tested and manufactured an instrument that enables consistent harvesting of good-quality PDEK tissue from donors of any age. This now has a CE mark. We were able to understand the science behind the structure and separation of PDEK tissue from the rest of the cornea and also advanced knowledge of the structure of the substance (stroma) of the cornea. This knowledge has benefitted understanding of DALK and EK.

GRANT REPORT

(A) CLINICAL AND SCIENTIFIC SIGNIFICANCE OF ADVANCES MADE

Full-thickness corneal transplant surgery has three major associated risks: (i) a weak graft–host junction that is vulnerable to rupture with trivial trauma even years after surgery; (ii) graft failure due to endothelial-cell rejection; (iii) induced astigmatism, cornea warpage related to sutures and scarring causing poor focussing and reduced visual benefit despite a clear graft.

Selective replacement of the diseased layer(s) can be achieved using DALK (for stromal disease) as well as DMEK and PDEK (pre-Descemet's endothelial keratoplasty) for endothelial disease. These procedures address the problems associated with corneal transplantation and are a major advance over full-thickness keratoplasty.

PDEK was developed after discovery of the PDL. It is a type of EK that gives as good vision as DMEK but offers some advantages. PDEK tissue, unlike DMEK tissue, can be harvested even from very young donors, which bring with them many endothelial cells and higher-quality endothelia. PDEK tissue is easier to handle and spread open in the eye. Endothelial cells in DMEK tissue are lost upon spreading in the eye, and this procedure takes longer or requires multiple manipulations. The learning curve for DMEK is very steep. Increasingly, PDEK is being done in multiple centres and offers a viable option for EK.

We wished to improve the scientific knowledge and understanding of PDEK, and make the procedure more accessible and consistent, especially with regard to obtaining PDEK tissue from donor eyes.

The dynamics of a 'big bubble' (BB) (i.e., separation of PDEK tissue) in donor eyes was studied in detail by simulating the *in vivo* procedure of DALK in human donor eyes and by following the path taken by air from the point of injection in the stroma to formation of a BB. This was tracked by videography, histology, light microscopy and electron microscopy. Injected air followed a consistent pattern: initially as radial tracks to the limbus, then as circumferential bands along the limbus, and finally centripetally to create predominantly a type-1 BB (i.e., separation of the PDL from the posterior stroma). Much less frequently, a type-2 BB (separation of the Descemet's membrane (DM) from the PDL) was formed. This type of BB started at the periphery of the cornea, which prompted investigation of the point of start of the type-2 BB. This led to the discovery of clusters of tiny fenestrations in the periphery of the PDL. Also, 15–20 such clusters were seen in control samples on either side of Descemet's attachment. Discovery of these fenestrations

was a novel addition to the microanatomy of the cornea. Air emerging through such fenestrations located central to the termination of the DM explained the type-2 BB. A type-1 BB was formed by air emerging through wide spaces between lamellae of deep stroma.

Histologically, the circumferential band revealed aggregation of air pockets in the mid-stroma. The consistent pattern of air passage is indicative of the architecture and microanatomy of the corneal stroma, where collagen lamellae are arranged orthogonally and centrally and as a circular annulus at the periphery. The novel peripheral fenestrations explain peripheral commencement of a type-2 BB and the escape of air into the anterior chamber during DALK. When air escaped from the fenestrations distal to termination of the DM, it escaped into the anterior chamber or outside the cornea *in vitro*. These results added to the knowledge base of corneal stromal anatomy, the separation of DMEK and PDEK tissue, and understanding of DALK in humans.

The only method for separating PDEK tissue is by air injection in the stroma and creation of a type-1 BB. There are two major issues with this method. The inconsistent and variable escape of air from the peripheral fenestrations makes it difficult to maintain constant intra-tissue pressure, thereby requiring the surgeon to adjust the pressure and rate of air injection to compensate for lost air. Too rapid injection results in burst (and loss) of PDEK tissue. Second, formation of a type-2 BB separates DMEK tissue, forcing the surgeon to undertake DMEK instead of PDEK, which he/she may not be comfortable with.

Knowledge of the fenestrations, mechanism of air escape, and formation of a type-2 BB led to the design of the 'PDEK clamp'. This instrument was used to clamp the periphery of the corneal disc at a diameter of 9 mm and shut out all fenestrations. The side port of the clamp allowed insertion of a needle and air injection, while its long handle allowed the surgeon to hold it in one hand and keep it steady during needle insertion and air injection. This clamp is now manufactured and CE-marked, and is being used for PDEK. It has made harvesting of PDEK tissue easy and consistent, encouraging surgeons to take on this procedure.

Some surgeons use injection of a viscoelastic substance instead of air for DALK and suggest its use for retrieving PDEK tissue. We explored use of a viscoelastic substance for these purposes and discovered a novel occurrence of substantial clinical relevance. Whereas air can be injected at any level of the stroma to obtain separation of PDEK tissue, a viscoelastic substance behaves very differently depending on the level of injection. Anterior stromal injection resulted in only some lamellar separation without BB formation. Mid-stromal injection often resulted in formation of an intrastromal BB, which has hitherto not been described. This has important implications for DALK and PDEK in that use of a viscoelastic substance is much less consistent in separating PDEK tissue and can mislead the surgeon into thinking that the desired separation has been obtained. The same effect was seen with different concentrations and volumes of a viscoelastic substance.

(B) PROBLEMS ENCOUNTERED AND STEPS TAKEN TO OVERCOME THEM

Significant problems were not encountered. Initially, we had some difficulty finding a suitable organisation to develop the idea and market it. We were fortunate in finding such an organisation, e.Janach, who took this through to manufacturing and obtained a CE mark. We had to work our way through three iterations of the prototype to perfect it.

(C) COLLABORATIONS ESTABLISHED

The instrument-manufacturing company e.Janach continues to work with us to develop other devices to enhance corneal transplant surgery.

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

- Dua HS, Faraj LA, Kenawy MB, et al. Dynamics of big bubble formation in deep anterior lamellar keratoplasty by the big bubble technique: *in vitro* studies. *Acta Ophthalmol* 2018;96:69–76.
- Dua HS, Said DG. Pre-Descemet's endothelial keratoplasty: the PDEK clamp for successful PDEK. *Eye (Lond)* 2017;31:1106–1110.
- Ross AR, Said DG, El-Amin A, et al. Deep anterior lamellar keratoplasty: dissection plane with viscoelastic and air can be different. *Br J Ophthalmol* 2018; pii:2017–311349.

(E) ACKNOWLEDGEMENTS

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King James IV Professorship Lecture 2016

Screening for developmental dysplasia of the hip

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Delivered at the British Orthopaedic Association Congress, Belfast, 15 September 2016

INTRODUCTION

The UK guidance for screening for developmental dysplasia of the hip (DDH)/congenital dislocation of the hip (CDH) was produced first by the Standing Medical Advisory Committee[1]. Risk factors stated to be associated with CDH were: caesarean section, foot deformities (fixed and postural), intrauterine growth restriction, family history, breech presentation and oligohydramnios. There was no evidence base validating the statement that 60% of CDH cases were associated with these risk factors.

Shipman and colleagues stated that "An effective screening programme must identify the cases of DDH earlier than would have been identified in the usual course of care and must lead to better functional outcomes than late treatment; any benefit should outweigh the harms of screening"[2]. DDH does not meet this aspiration or most of the criteria for an 'ideal' screening programme (Figure 1). Several important World Health Organization criteria are not met: the natural history of the condition is not known; there is no scientifically proven effective early treatment; opinions on who should be treated are controversial; there is no recognisable latent and early symptomatic stage; the sensitivity and positive predictive value (PPV) of clinical screening tests are poor.

CDH was renamed DDH in 1989[3] upon recognition that not all cases of pathological hip conditions associated with DDH were present at birth. DDH is a dynamic condition in which the hip abnormality may improve or deteriorate with growth. It has a spectrum of presentation varying from hip dysplasia, to reducible subluxation/dislocation and, eventually, irreducible dislocation of the hip joint[3]. Neurological and neuromuscular syndromes and skeletal dysplasias are excluded because the hip abnormality is secondary to a primary disease and is not idiopathic[4]. The traditional outcome measure is irreducible dislocation of the hip[5].

The development of sonographic imaging of the hip joint has blurred the diagnosis of what constitutes a 'true' pathological disorder of the hip. A clinical Ortolani and/or Barlow positive hip manoeuvre will resolve spontaneously in 70–90% of cases within 2–4 weeks post-natally[6, 7]. Sonographically, 90% of Graf type-II dysplasias, <25% of Graf type-III dysplasias and <90% of Graf type-IV dysplasias may resolve[8–12].

The diagnosis of hip disease in DDH screening may be by clinical examination and/or by sonographic imaging. In the UK, the recent screening policies have been universal neonatal clinical screening of hip joints at birth (Ortolani and Barlow manoeuvres) and a general practitioner (GP)/healthcare professional assessment at 6–8 weeks[1,13]. The underlying problems with such clinical hip-screening tests are that the Ortolani manoeuvre is only 60% sensitive and the Barlow manoeuvre has a PPV of only 22%[14–16]. The Barlow and Ortolani manoeuvres fail to identify 66.7% of hip joints that require surgical intervention subsequently[17]. Sonographic screening of hip joints may be universal in neonates or selective 'at risk' screening at 6 weeks of age. A sonographic diagnosis of DDH has a higher prevalence of abnormality than a clinical diagnosis of hip instability, raising the possibility of over-diagnosis of pathological hip conditions, which may lead to over-treatment.

Due to these issues, the efficacy of clinical hip-screening programmes in the UK and USA has been disputed[14–17]. The prevalence of 'late' or overall irreducible dislocation varies worldwide from between 0.07 to 0.5 per 1000 live births[18–22] and may be affected by various genetic and local environmental factors. Review of the literature on DDH screening shows that national/international levels of evidence are poor and that studies are mainly uncontrolled and observational[24]. Conclusions drawn from such limited research must be guarded. In the USA, a systematic review assessed nearly 4000

articles on DDH screening and concluded that in only 32 articles was the structure and content of the research of adequate scientific quality to produce meaningful clinical guidance. There were only two areas in which the evidence in the research was considered to be of 'moderate' strength: (i) rejection of universal ultrasound screening; (ii) undertaking an imaging study (ultrasound or radiography) <6 months of age for a patient with a strong family history of DDH, for a breech presentation, or a history of clinical instability[25].

In 2004 and 2008, the hip-screening policy was updated in Newborn, Infant Physical Examination (NIPE) guidelines[26]. The main recommendations from NIPE were that the Ortolani and Barlow manoeuvres should be undertaken within 72 h of birth and, if abnormal, the hip joints should undergo ultrasound within 2 weeks. If there is a significant abnormality on ultrasound then expert opinion should be sought by 3–4 weeks. In addition, the at-risk hips of a breech presentation and a strong family history merits hip ultrasound scanned within 6 weeks of the birth. If this scan is abnormal the infant should be given an expert opinion to decide on the treatment plan by 6–8 weeks of age. In infants that have been passed normal at birth with no risk factors, there should be a clinical hip-joint assessment by a GP/healthcare professional at 6–8 weeks.

In 2016, Public Health England updated NIPE guidelines adding certain 'screen-positive signs' at or after the assessment at 6–8 weeks that required expert referral. These screen-positives signs were asymmetrical skin creases (ASCs), limitation of abduction (LHA) <90°, a positive clunk on the Ortolani manoeuvre, and a positive Galeazzi assessment of femoral shortening. The first two screen-positive signs (bilateral LHA and ASC) are controversial clinical signs because their sensitivity and PPV are thought to be poor.

Worldwide, there are no agreed guidelines or standards. In Austria and Germany, there is a universal screening programme for neonatal hip joints using ultrasound. In Switzerland, universal hip screening using ultrasound was abandoned in 2004 because the evidence base was too poor. In other countries, there is a spectrum of policies ranging between universal hip screening with or without selective hip screening using ultrasound[2, 18, 22, 23, 20, 25, 27, 28, 29].

For neonates, a significant difference has not been found in outcome when comparing universal clinical examination alone, universal clinical examination with selective ultrasound hip screening, and universal clinical examination with universal ultrasound hip-screening methods [28, 29].

There are numerous ultrasound classification systems lacking validation and with poor kappa scores[18, 30, 31, 33]. Ultrasound abnormalities could be considered to be a driver of over-diagnosis in DDH. Black stated that the "ability to detect smaller abnormalities axiomatically tends to increase the prevalence of any given disease"[32].

Personal research: DDH screening

My doctoral thesis[33] focused on the three main hip-screening pathways in the UK: neonatal clinical hip screening; selective at-risk ultrasound screening; clinical hip assessment by a GP at 6–8 weeks. Other longitudinal observational cohort studies reviewed included: limitation of hip abduction; congenital talipes equinovarus (CTEV); risk factors of a breech presentation; family history in clinically stable hips and their association with pathological DDH[34–37]. An audit into the importance of ASCs was undertaken in 2016.

Results and discussion

Statistical analyses of these screening pathways from my doctoral thesis[33] showed that primary clinical neonatal screening had a sensitivity of 66%, specificity of 99.77%, and a PPV of 27.97%. In selective at-risk ultrasound screening, the sensitivity was 100%, specificity was 94.22% and the PPV was 20.47%. Using ultrasound, if stable Graf type-III dysplasia was excluded, the specificity was 93.3% and PPV was only 7.02%. Clinical screening by a GP had a sensitivity of 13.34%, specificity of 99.9%, and PPV of only 4.65% (Figure 2). The data for clinical neonatal screening (sensitivity and specificity) were very similar to the results of the studies by Jones in the 1970s and 1990s[14,15]. The PPV also confirmed that 70–80% of potential clinical hip instabilities resolved spontaneously. There was a higher prevalence of pathological hip abnormalities diagnosed by ultrasound methods compared with clinical assessment, the true significance of which has not been established.

If clinically unstable hips presenting with at-risk factors were excluded and stable at-risk hips alone were assessed, the incidence of pathological hips (sonographic Graf type-III, Graf type-IV, and radiological irreducible hip dislocation) in breech presentations in males was 1:594 and in females was 1:32; in those with a strong family history, in males it was 1:99 and in females it was 1:28. The previously considered 'risk factors' of postural talipes equinovarus (TEV), oligohydramnios, CTEV and clicky hips did not appear to be true risk factors in the development of pathological DDH.

Statistical analyses showed that the 95% confidence intervals of the mean (95% CI) were tight in CTEV, congenital talipes calcaneovalgus and breech presentation, confirming that the diagnostic criteria were robust. However, the 95% CIs for family history, oligohydramios, and postural TEV were wide, thereby questioning the accuracy of the diagnostic data. Many of these conditions are 'soft' diagnoses open to interpretation and inaccuracy.

In a 10-year longitudinal observational study[36], 492 patients were diagnosed with limitation of hip abduction with a subset of 55 infants with unilateral hip abduction. The PPV was 40% for unilateral hip abduction compared with a PPV of 0.3% for bilateral hip abduction (outcome measure for pathological DDH: Graf type III and IV or irreducible hip dislocation). The extremely low PPV of 0.3% for bilateral limitation of hip abduction made effective clinical screening for bilateral irreducible hip dislocation impossible. In unilateral limitation of hip abduction, if this assessment was undertaken at ≥8 weeks of age, the sensitivity was 78.3% and PPV was 54.7%, so this was a time-dependent association.

There were no cases of persistent pathological DDH or hips requiring a Pavlik harness/casting or surgical procedures in 199 feet presenting with fixed CTEV[35]. In 982 cases of males born by breech presentation without signs of clinical instability, there were no cases of pathological DDH. There was a significant difference between the incidence of pathological hips (sonographic diagnosis) in 1360 males (0.003) and 1624 females (0.028) ($p<0.001$) in cases presenting with stable hip joints clinically with the risk factors of breech presentation or a strong family history[37]. Also, 74% of Graf type-IV hips and most irreducible hip dislocations (94.4%) were in females[34]. Most irreducible hip dislocations (58% of the total of irreducible hip dislocations) were not identified from the screening group looking at neonatal hip instability by a GP[38]. This is very similar to the prevalence of late-presenting irreducible hip dislocation before selective sonographic hip screening[17].

An audit in 2016 evaluated cases referred with ASCs over a 21-year period. There were no cases of pathological DDH in those with ASC alone (no associated limb-length discrepancy or limitation of hip abduction).

Conclusions

Six main conclusions can be drawn.

- The current hip-screening programme is not effective and should be considered surveillance only.
- Addition of sonographic-selective screening of all at-risk hips to universal clinical hip screening has not reduced the prevalence of late-presenting irreducible dislocation.
- The GP hip-screening examination is ineffective in its present form.
- Spontaneous resolution of clinical hip abnormalities makes the diagnosis of true pathological hip dysplasia difficult.
- The natural history of clinically normal but sonographic hip-joint abnormalities is not clear.
- Pathological DDH is mainly a female condition and most males are not 'truly' at risk if the hip joints are clinically stable.
- Most previously accepted risk factors are not truly risk factors of pathological DDH.

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The condition should be important*
The examination and/or treatment are/is acceptable to the patient*
Continuously rolled out and repeated*
Treatment and diagnostic facilities should be available*

There should be an effective and accepted treatment
Recognisable latent and early symptomatic stage should be present
Opinions on who should be treated are agreed

The natural history of the condition should be known

Guaranteed safety, sensitivity and specificity of the test (ideally >90%)
Tests are inexpensive and simple
Cost-effective programme

Those marked * are criteria present in the DDH screening programme

After Wilson & Jungner 1968

Figure 1: 'Ideal' screening criteria for DDH.

	Primary neonatal screening (clinical)	Primary neonatal screening (sonographic)	'At risk' hip screening (sonographic)	'At risk' hip screening (sonographic) Graf type-III hips removed	Clinical check at 6–8 weeks by GP
Sensitivity	66%	72.07%	100%	–	13.34%
Specificity	99.77%	99.90%	94.22%	93.3%	99.9%
Positive predictive value	27.97%	67.8%	20.47%	7.02%	4.65%
Negative predictive value	99.95%	99.92%	100%	–	99.96%

Figure 2: Statistical analyses of screening pathways.

King James IV Professorship Lecture 2017

Transforaminal lumbar endoscopic discectomy and decompression: review of methods and supporting evidence

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International Society for the Study of the Lumbar Spine (ISSLS)
44th Annual Meeting, 29 May to 2 June 2017, Hilton Hotel and Conference Centre, Athens

INTRODUCTION

I was most grateful to the ISSLS Secretariat for allowing me to present a plenary lecture at their 44th annual conference. The purpose of the ISSLS (a non-profit organisation founded in 1974) is to bring together individuals who, by their contributions and activities in research and clinical study, have an interest in the lumbar spine in health and disease. Its further purpose is to serve as a forum for the exchange of information of an investigative and clinical nature which relates to low back pain and disability. ISSLS has about 270 active members from all nations elected by invitation. The congress was attended by 700 international delegates.

SUMMARY OF LECTURE

In the last 20 years, there has been a revolution in spinal surgical care with a drive towards achieving decompression of the neural elements and stabilisation of the unstable spine with minimal insult. Surgeons recognise that open approaches to the spine require extensive soft-tissue stripping that would devitalise the structures providing spinal support. Since 2006, I have been undertaking various forms of transforaminal endoscopic lumbar spinal surgery to minimise muscle injury and enhance patient recovery. A description of these methods and evidence from our studies in Edinburgh form the basis of this lecture.

One of the conditions of ISSLS is that each presentation of a new surgical method is counterbalanced by an opposing view. This was provided by Miss Helena Brisby (Stockholm): "When are 'open' spine techniques for the lumbar spine preferred?"

1. Relevance in terms of muscle injury

Formation of epidural scar tissue following spinal surgery is part of natural healing, and is frequently associated with failed back surgery syndrome[1]. Aside from contributing to poor surgical outcome, epidural fibrosis leads to an increased prevalence of complications in revision spinal surgery[2,3]. Minimising scarring and damage to the paraspinal muscles by minimally invasive approaches is ideal provided that methods are safe and the end outcomes are equivalent to those from traditional open methods[4].

2. History of transforaminal endoscopic spinal surgery (TESS)

In 1990, Kambin described a 'safe-working' zone allowing endoscopic access *via* a transforaminal approach to the lumbar spine. Since then, surgeons and instrument manufacturers have sought to facilitate surgery by improving visualisation of the disc and neural structures by careful attention to lumbar anatomy[5,6].

3. Surgical method

The method of transforaminal endoscopic discectomy has been described with reference to my publication in *The Surgeon*[7].

4. Evidence supporting transforaminal approaches to the spine

The literature with respect of minimally invasive surgery to the lumbar spine, including Cochrane Reviews and meta-analyses, were reviewed[8–10]. The results preceded those from the Edinburgh randomised controlled trial and cost-effective analyses of TESS[11,12].

5. Discussion regarding interlaminar endoscopic spinal surgery

Applications of this procedure as an alternative to TESS at L5 have been discussed with a description of the surgical method and a representative case study.

6. Description of transforaminal endoscopic foraminotomy
The instrumentation required and results from the Edinburgh series presented in San Francisco in 2015 have been outlined[13].

7. Description of excision of cysts in transforaminal facets
Results from the Edinburgh series, as presented initially at Spine Week in Singapore in 2017, have been discussed[14].

8. Analyses of a surgical case demonstrating the relative cost of various spinal procedures
Different treatment options have been put forward for a complex case.

9. Possibilities of surgical decompression using endoscopic approaches
The place for interlaminar endoscopic decompression using powered reamers for the relief of spinal stenosis has been described with a case presentation[15].

10. Transforaminal endoscopic spinal fusion
This section includes a description of surgical methods and early results of the Edinburgh Series. The work was presented at the 2017 Eurospine meeting in Dublin.

11. Future developments in endoscopic surgery
The lecture concludes with descriptions of potential advancements in TESS using three-dimensional imaging, robotic control and holography.

The lecture video is available at:
www.youtube.com/watch?v=xQ0_CWLnFuI&t=46s

and counter-lecture is at:
www.youtube.com/watch?v=mhz76dFl1eI&t=19s

In the last 6 months, four articles have been published or submitted:

- Gibson JNA, Subramanian A, Scott CEH. A randomised controlled trial of transforaminal endoscopic discectomy versus microdiscectomy. *Eur Spine J* 2017:26:847–856.
- Scott CE, Gibson JNA. Letter to the editor concerning "A randomised controlled trial of transforaminal endoscopic discectomy versus microdiscectomy". *Eur Spine J* [submitted].
- Bucknall V, Gibson JNA. Cervical endoscopic spinal surgery: a review of the current literature supporting the technique. *J Orth Surg* [submitted].
- Middleton S, Wagner R, Gibson JNA. Multi-level spine endoscopy: a review of available evidence and case report. *Bone Joint J* [in press].

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King James IV Professorship Lecture 2017

Multimodal treatment of eye tumours – advances in chemotherapy, radiotherapy and surgery

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Eye tumours comprise intraocular, ocular surface, orbital and adnexal lesions. Such diseases include intraocular tumours in children and adults (e.g., retinoblastoma and uveal melanoma) and tumours on the ocular surface (e.g., ocular surface squamous neoplasia (OSSN)). These are rare tumours but have consequences not just for sight, but also for retention of the eye and life prognosis in many cases[1].

Retinoblastoma, the most common intraocular cancer in children, is a disorder initiated by mutation of RB1. Occurring in approximately 1 in 18,000 live births, 50 children per year are affected in the UK. Untreated, retinoblastoma is fatal by spread via the optic nerve to the central nervous system or through the bloodstream. A white pupillary reflex and strabismus are the most common presenting symptoms. In developed countries the prognosis for children with retinoblastoma is good, with 5-year survival >95%, and the focus now is on early diagnosis and retention of the eye with as much vision as possible.

During the 20th century the main conservative treatment was external-beam radiotherapy (EBRT) but this was associated with an increased risk of subsequent cancers in patients with genetic retinoblastoma. Systemic chemotherapy supplanted EBRT as the main treatment for intraocular retinoblastoma in the 1990s. EBRT was used mainly as salvage treatment when chemotherapy and local treatments (e.g., plaque radiotherapy, laser or cryotherapy) of tumours had failed, but is now regarded as tantamount to treatment failure[2].

New treatment modalities have evolved and a wide armamentarium is available to treat the five groups (A to E) of the International Intraocular Retinoblastoma Classification (IIRC). Group-A eyes (which have the least advanced tumours) are often amenable to treatment by focal methods (e.g., cryotherapy, laser thermotherapy) with very high success rates. Group-E eyes (which have the most advanced tumours) are usually removed by enucleation. The middle groups, B to D, are treated conservatively in most cases, though eventual globe retention in group D is ≈50% in eyes receiving systemic chemotherapy initially. Our recent work has shown eye retention in 63% of such eyes using multimodal treatments[3]. In that report, all cases were treated initially with systemic chemotherapy, as well as a median number of three other treatments (mean: 6; range: 0–24), including thermotherapy or cryotherapy, plaque radiotherapy, intra-ophthalmic artery chemotherapy (IAC) and/or intravitreous chemotherapy. EBRT was used in five eyes, all of which were enucleated eventually. There were no cases of metastatic spread from intraocular retinoblastoma and no deaths. This report showed that IAC has now replaced EBRT as successful salvage treatment.

With improved survival in developed countries, there has been an impetus to treat retinoblastoma without removal of the eye and to preserve vision. Advances in ocular drug delivery over the last decade are revolutionising treatment of this cancer. Recent developments have delivered chemotherapy into the ophthalmic artery (IAC) or directly into the vitreous cavity. Local treatment of retinoblastoma with chemotherapy is an attractive idea to avoid potential systemic complications, such as neutropenic sepsis or cumulative organ toxicities.

However, initial enthusiasm for IAC with melphalan was tempered with realisation of the side-effect profile of this treatment. The first report from our research team found 80% tumour control at a mean follow-up of 8.7 months, but also a 80% chance of side effects, such as palsy of the third cranial nerve in 40%, orbital oedema in 20%, permanent retinal detachment in 7%,

and vitreous haemorrhage in 27%[4]. Furthermore, in eyes that had potential for good vision, 42% demonstrated severe visual loss following IAC at a median follow-up of 21 months[5]. This was due to retinal detachment (20%) or choroidal ischaemia involving the foveola (80%). Technical difficulties or vasospasm during ophthalmic-artery catheterisation and a non-age-adjusted dose of melphalan were factors in visual loss though, in later work, the risk of vision loss could be mitigated using age-adjusted doses of chemotherapy drugs[6]. Also a different type of relapse following attempted salvage by IAC was found whereby retinal tumours could be brought under control, but could seed into the anterior chamber[7]. In 6 eyes (50%) anterior-chamber invasion was clinically detectable. On histopathologic examination of 12 enucleated eyes where IAC had failed, 33% had no viable retinal tumour; the remainder had a poorly or moderately differentiated tumour. Anterior eye involvement occurred in the ciliary body and/or ciliary muscle (58%), iris (50%), and cornea (33%). This is a potentially dangerous location in the eye because the outflow of the aqueous humour through the trabecular meshwork could act as a portal for metastases. Most group-E eyes with retinoblastoma are enucleated, and groups A to C have smaller tumours with a low risk for metastatic potential. It is group D that can pose the biggest therapeutic challenge because valid treatments include enucleation or eye salvage with systemic chemotherapy or primary IAC. Some eyes undergoing an attempt at conservative therapy may have high-risk histopathologic features, increasing the risk of metastatic disease if IAC is considered first-line treatment. In our series of group-D enucleations, 13% of these eyes harboured high-risk histopathological features at presentation, with the absence of vitreous seeds being a potential risk factor[8]. Therefore, it may be safer to treat group-D eyes with vitreous seeds with IAC. Trying to save a group-D eye poses a burden of disease not just in the number of treatments required to keep the eye (as opposed to one curative procedure to remove the eye) but also in the number of times anaesthesia must be administered between the two groups. At a median follow-up of 61 months, patients with primary enucleation had on average 7 examinations under anaesthesia and chemotherapy-treated patients 21 examinations under anaesthesia ($p<0.001$)[9]. An older child with a unilateral group-D eye carries a negligible risk for the fellow eye to develop retinoblastoma – this is the most suitable group for primary enucleation of a group-D eye.

Other rare tumours in paediatric eyes include medulloepithelioma, which originates in the non-pigmented ciliary epithelium. These can be teratoid or non-teratoid, and benign or malignant. We have developed a method for treating such eyes by surgical plaque radiotherapy, rather than enucleation of the eye, to neutralise malignant elements within the tumour. There were 6 eyes with medulloepithelioma that presented to our research team in London during 7 years. Of these, 5 were selected for eye-sparing treatment by surgical placement of a ruthenium-106 plaque applicator under general anaesthetic on the globe surface directly over the tumour, and delivered 40–50 Gy to the tumour apex. To date, all of these eyes have been retained with regression of the mass in 80%, and visual acuity improved in a similar number[10].

In adults, the main intraocular primary tumour is uveal melanoma, with 500–600 cases per year in the UK. The US Collaborative Ocular Melanoma Study (COMS) was an example of a randomised controlled trial for a rare disease. From the 1980s to the present day, COMS reports have shown that conservative treatment of uveal melanoma with radioactive brachytherapy plaque applicators has equivalent mortality to enucleated cases. However, any melanoma next to the optic nerve (juxtapapillary melanoma) was ineligible for inclusion in the COMS plaque radiotherapy trial and instead offered enucleation. In collaboration with Dr Jerry Shields and Dr Carol Shields of the Ocular Oncology Service of Wills Eye Hospital, Philadelphia, a detailed analysis was undertaken of outcomes of iodine-125 plaque radiotherapy for juxtapapillary melanomas. In this series of reports in 650 cases over 31 years, Kaplan–Meier estimates for tumour recurrence, metastasis, and death were 21%, 24%, and 9%, respectively, at 10 years[11], and visual acuity of 20/200 or worse occurred in 87%[12]. Even if the tumour was completely overhanging[13] or encircling[14] the optic nerve, tumour control was >85%. Some eyes are enucleated as a secondary event due to non-response to radiotherapy or complications such as painful neovascular glaucoma[15]. Insertion of an orbital implant to rehabilitate the socket using a method that avoids tissue restitution prevents implant extrusion[16].

On the ocular surface, the main therapeutic option of tumours such as melanoma and OSSN is surgery. OSSN in temperate climates is found in elderly men who have been sun-exposed. It is encountered far more commonly in sub-Saharan Africa, often in young adult females due to infection by the human papillomavirus or human immunodeficiency virus as well as continuous exposure to sunlight[17–21]. Following tumour resection, there is a high risk of recurrence which, if sufficiently severe, may

require exenteration of the orbital contents to achieve local tumour control. In developed countries, several interventions can reduce recurrence risk, from simple cryotherapy at the time of initial excision to the residual margins, to use of chemotherapy drops or immune modulatory drops, to plaque radiotherapy. In developing countries, access to these options is not readily available – many operating theatres do not have access to cryotherapy and topical immune drugs are costly. In Kenya, Gichuhi *et al.* reported the results of a randomised controlled trial of tumour excision with or without topical 5-fluorouracil (an inexpensive chemotherapy drop) to prevent recurrence[22]. Results showed that this simple intervention reduced recurrence prevalence from 36% in the no-adjuvant-drops arm to 11% in the topical-5-fluorouracil-arm at 1 year, with an adjusted odds ratio of 0·23 (95%CI 0·07–0·75; p=0·02).

In ocular oncology, a wide variety of tumours are treated. Some of these are life-threatening whereas others threaten the globe or vision. For example, benign tumours such as choroidal naevi[23] or osteomas[24] can spawn choroidal neovascular membranes that cause blurred vision from serosanguinous retinal changes and which can be treated with intravitreal injections of anti-vascular endothelial growth factor to preserve sight. Overall, several treatments are required to manage intraocular and ocular-surface tumours, with important approaches from surgery, radiotherapy, chemotherapy to local treatments such as laser, cryotherapy, intravitreal treatments and topical chemotherapy.

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