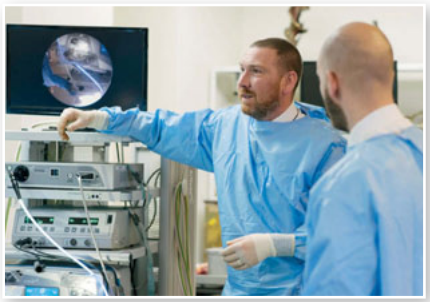
Orthopaedic Institute



ANNUAL REPORT 2022/23

Including:
Reports on research in collaboration with the Robert Jones and Agnes Hunt Orthopaedic Hospital NHS Foundation Trust and Schools of Pharmacy & Bioengineering and Medicine, Keele University, Staffordshire

www.orthopaedic-institute.org

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INTRODUCTION

The Orthopaedic Institute Limited founded in 1971, (**www.orthopaedic-institute.org**) is a registered national charity and through voluntary contributions, helps to fund research in the specialist research centres and departments within the Robert Jones and Agnes Hunt Orthopaedic Hospital NHS Foundation Trust in Oswestry, Shropshire. It also supports the funding of educational facilities and activities such as organising training courses for orthopaedic trainees and allied health professionals.

The Executive Committee, under the Chairmanship of Mr Eric Evans, decides the policy of the Institute and monitors its progress. A Research Panel, under the Chairmanship of Dr Robin Butler considered new research projects proposed by research staff and clinicians, and recommended to the Executive Committee, the projects to be funded from the income provided by grants and donations received.

It is with great sadness that we have to inform readers that shortly before preparing this report, Dr Robin Butler passed away. Dr Butler was a Consultant Rheumatologist here for many years and lately took on the role of Chairman of the Research Panel. He will be sorely missed.

Our condolences and thoughts are with his family.

The research referred to in this Annual Report has been undertaken by the Research Departments, Institute of Orthopaedics, the Robert Jones and Agnes Hunt Orthopaedic Hospital and Schools of Pharmacy & Bioengineering and Medicine at Keele University, which all work in close collaboration.

This includes projects funded by the Orthopaedic Institute Ltd and projects for which funds have been obtained by the NHS Foundation Trust and external organisations.

Research staff employed on the projects are either supported by the Orthopaedic Institute or are working within Departments of the Hospital or Schools of Pharmacy & Bioengineering and Medicine at Keele University. Many programmes are interdisciplinary and involve staff from one or more departments, resulting in new and improved methods of treatment as well as pioneering additions to medical knowledge.

A full set of Accounts are available upon request, please contact **enquiries@orthopaedic-institute.org** for further information.

Registered Charity Number: 1044906

CHAIRMAN'S REPORT

For over 50 years the Orthopaedic Institute has funded research and education to improve the treatment of patients with conditions that affect the musculo-skeletal system at the Robert Jones & Agnes Hunt Orthopaedic Hospital Foundation Trust, a long-standing centre of excellence in world orthopaedics.

It is pleasing to report that although research studies and teaching were severely affected by the pandemic, both areas are now fully restored to full capacity and continue to develop with many innovative research projects and more training courses being held, funded through us by external Charitable Trusts, Foundations, patients and members of the public. We are extremely grateful for the support received year on year which enables us to continue with these vital services.

It is also pleasing to see that many research initiatives instigated with the support of the Orthopaedic Institute are thriving and now in their post-research phase, have been integrated into the core clinical work of the hospital.

Administration

There were no changes in the Board of Directors during the current year.

The financial standing of the Institute remains sound and we are indebted to our treasurer Louise Osselton and bookkeeper Fiona Bain for their careful management of our resources. The Board keeps a watch on the management of our investments and is satisfied with the current performance.

Judy Harris continues to provide administrative support to the board and we are grateful for the care and attention that she brings to the post.

We welcomed our new Course Organiser, Laura Haythorn during the year. Laura has worked hard in effectively reinstating and bringing in a number of new courses working with our clinical colleagues, after the inevitable disruption and restrictions caused by the pandemic. Ongoing continued development of our flagship courses is progressing to reflect the changes in Postgraduate medical education in the NHS. Laura will work with our trainers to ensure that Oswestry maintains its renowned reputation.

Debra Alexander has had a successful, albeit challenging year in her role as Fundraiser. Again, the pandemic severely restricting fund-raising activities. The Annual Snowpaedic Challenge, a sponsored hike up Snowdon for ex-patients, staff and supporters, was held in September although with fewer participants than we have previously enjoyed. Donations to support research projects and items of laboratory equipment have continued and we are grateful to the external Trusts, Foundations and Charities that have supported our requests.

Research

Many thanks go to Dr Robin Butler for standing in as Acting Chairman of the Panel who stood down for personal reasons during the year. We welcome Teresa Jones as the new Chair. Teresa comes with wealth of knowledge gained during her previous career in Research Management. The Panel continues to perform its duty of providing peer review for projects submitted for funding and monitoring their progress.

Throughout the year 2022-2023 the Institute has supported a number of research projects, with many more in the pipeline. The range of research is impressive, varying from clinical trials in common orthopaedic problems to molecular biology approaches and to less common forms of muscular dystrophy. Also, from radiological studies on patients with back pain to gait analysis in children with cerebral palsy. The unifying factors are that they are all well designed studies on conditions affecting patients attending our hospital.

We have also provided funding to purchase and maintain new pieces of laboratory equipment and awarded 24 grants to cover a variety of specialist training courses, publishing fees and placements for upcoming PhD student 'scientists' of tomorrow.

Research Day held on Friday 27th April in tandem with the Specialist Orthopaedic Registrars was a stimulating day and our thanks go to all who contributed.

Teaching

The Institute has started again to convene courses in the review period after our enforced furlough and we are grateful to the convenors and faculty of each for the work they put in.

Our flagship courses, the Clinical Examination and Viva Courses, have earned world-class reputations and delegates consider them to be the 'best of their kind' and as new delegates were eager to book, we were pleased to run the Clinical Examination course again in October.

The reputation of the courses has been built on the participation of a huge variety of model patients and the commitment of the highest quality Lecturers and Examiners and we are grateful to all involved.

Current Projects

The Institute has excellent relations with the leadership team of the RJAH Foundation Trust and is working with them, within its charitable aims, to take forward important initiatives for the future.

Veteran's Outpatient Centre

The Headley Court Veteran's Orthopaedic Centre for which the Orthopaedic Institute provided the initial impetus and funding, was opened by Sophie, the Duchess of Edinburgh in May 2023 having been supported by the Headley Court Charity. We are very proud to hold the Bronze Award for the support we provide to our Veteran's.

Cell Therapy Unit

Fully financed by the Orthopaedic Institute over twenty years ago, the Oscell Cell Therapy Unit provides cells for clinical trials to repair damaged joint cartilage. Changes to the regulatory framework under which cells are produced have required modification and extension of the facility. The Institute has supported this and a numbers of studies and will continue to assist in the long-term development of cell therapies.

Education/Training Centre

The Institute has worked with the hospital to upgrade our current Lecture theatre and Library, but it is clear that a new centre will be needed in the near future to allow an expansion of training for all clinical specialties. The Institute funded an options review, being the first stage of a business plan for the project. We look forward to supporting this venture as ideas to maximise the number of courses and training available to colleagues and allied professionals from across the globe progresses.

In conclusion

This report provides a flavour of the innovation and precision of the research being carried out by the clinicians and scientists at the Robert Jones & Agnes Hunt Orthopaedic Hospital NHS Foundation Trust.

The Orthopaedic Institute provides the financial support sought by researchers to get projects started and through to proof of concept phase. We also play an important role in providing the technology to keep our researchers at the cutting edge.

Please help us help them – together we have a great record of success.

Eric Evans
Chairman
Orthopaedic Institute Ltd

METABOLIC BONE CENTRE

Metabolic Bone Clinical Service
Clinical Lead: *Dr Chadi Rakieh*

The metabolic bone service has members of staff working in the laboratory, the bone density unit, the day case unit as well as patient clinics. Staffing is made up of a consultant, speciality doctors, specialist nurses, clinical scientist, metabolic technician, radiographers, DXA technician and HCAs. In 2022 we scanned over 8800 patients and over 1400 patients received intravenous treatment for their bones.

In the laboratory over 1800 patient samples were analysed for the bone resorption marker urinary N-telopeptide crosslink of type I collagen (uNTx), a long-established marker of bone breakdown as part of the clinical service. The marker is used to monitor treatment efficacy, monitor treatment holidays and to determine baseline bone turnover.

Funding from RJAH charitable funds in 2019 allowed us to instal an IDS (Immunodiagnostic Systems Ltd) iSYS speciality automated immunoassay analyser see Figure 1. We currently use this to measure the bone formation marker PINP (total procollagen type-1 N-terminal propeptide) in patients attending our outpatient clinics (plasma from a blood sample). Measuring this marker gives us a more complete assessment of bone status in our patients and improves the precision of our clinical decisions including assessing patients on anabolic therapy (parathyroid hormone), whose use is growing in clinical practice. We measured just over 1000 samples for PINP in 2022 and 2750 in total by the end of 2022.

We presented data at the Osteoporosis Online Conference in December 2020 on the use of PINP below the premenopausal reference mean as a useful treatment target for patients on anti-osteoporosis treatment [1]. We showed that 87% of untreated patients with osteoporosis had a PINP level above this target while only 7% of patients treated with IV bisphosphonate were above the treatment target.

Figure 1. Diane Powell loading the automated analyser with patient samples.

With funding from the Orthopaedic Institute, we were able to measure a reference range of pre- and post-menopausal women and of men to compare our local population with that of other groups. We had been using the manufacturer's reference range to identify patients with high PINP levels but the reference range was much wider than other published reference ranges. We collected data from a total of 62 volunteers – 20 males aged 50-77 years, 21 premenopausal females aged 30-45 years and 21 postmenopausal females aged 50-61 years. Samples were collected in plasma in a non-fasting state. As described in the CSLI guideline C28-A2 a previously established reference range can be validated using 20 results from a new laboratory using the same technique.

The results were compared to the published reference intervals and considered valid if no more than 2 out of the 20 tested values fell outside the original stated reference interval limits.

Due to reduced pre-analytical variation in PINP the differences between the previously published reference intervals and our study population did not result in significant differences in PINP levels. This meant that the reference intervals can effectively be transferred to our population and no further analysis was required. This means we now use a more specific reference ranges for male, pre- and post-menopausal females.

We also presented data on bone microarchitecture in liver cirrhosis at the Osteoporosis Online Conference [2]. We can analyse data from the bone density scans of the lumbar spine to produce a trabecular bone score (TBS) which is an index of microarchitecture and is validated as an independent risk factor for fracture. We were able to show that patients with liver cirrhosis had a lower TBS score than controls matched for age, sex, BMI and lumbar spine bone density. Compromised bone quality may have an important role in the aetiology of metabolic bone disorder associated with chronic liver disease.

Published Abstracts:

1. Powell DE, Evans SF, Rakieh C (2020) Evaluation of treatment target response in real-world clinical service. Therapeutic Advances in Musculoskeletal Disease 12; 7-8.
2. Rakieh C, Silva S, Roberts T, Powell DE, Ho S, Butler R (2020) Trabecular bone score as a risk factor of bone disease in liver cirrhosis. Therapeutic Advances in Musculoskeletal Disease 12; 40-41.

RHEUMATOLOGY RESEARCH

Members of the Research Team:
Dr Oksana Kehoe (Lead) and PhD students Rebecca Davies, Anais Makos and Henry Barrett

Clinical Support:
Dr Roshan Amarasena (Lead), Dr Ayman Askari, Dr Julia Flint and Dr Rameez Arif.

Our team has continued to be active this year carrying out basic science work into understanding mechanisms of inflammatory arthritis diagnosis, progression and possible treatments including mesenchymal stem cells, regulatory T cells and their extracellular vesicles. We also try to find out how stem cells can be "encouraged" to perform better in aging and in disease such as arthritis.

We are very fortunate in RJAH to be able to work closely with clinicians who themselves are very interested in research.

We have been publishing our findings in the *International Journal of Molecular Sciences, Inflammopharmacology, Cytotherapy and Annals of the Rheumatic Diseases* and have presented our work at several national and international meetings such as the 8th Malaysian Tissue Engineering and Regenerative Medicine Scientific Meeting, March 2022; the International Society for Extracellular Vesicles Annual Meeting, May 2022, Lyon, France (Figure 1); TERMIS EU 2023, March 2023, Manchester, UK; the International Society for Extracellular Vesicles Annual Meeting, May 2023, Seattle, USA; EULAR 2023 Congress, May 2023, Milan, Italy.

Figure 1. Dr Oksana Kehoe and Becky Davies at the ISEV Annual Meeting in Lyon, France, May 2022.

We are very grateful to the Orthopaedic Institute Ltd, Oswestry for their continuous support.

The projects on which we are currently working are explained in a little more detail as follows:

SCALING UP MESENCHYMAL STROMAL CELL EXTRACELLULAR VESICLE PRODUCTION FOR THERAPEUTIC APPLICATION IN RHEUMATOID ARTHRITIS

Rebecca Davies, Dr Claire Mennan, Mark Platt, Dr Karina Wright, Dr Oksana Kehoe

Funded by the Orthopaedic Institute James Richardson Studentship, Keele University and EPSRC/MRC Centre for Doctoral Training in Regenerative Medicine

We work with the goal of finding effective therapies for rheumatoid arthritis (RA), hoping to combat the inappropriate activation of the immune system at its root. One potential therapy involves the use of a certain type of cell, mesenchymal stromal cells (MSCs), that show great promise in restoring balance to a wayward immune system. Unfortunately, cell therapy is not as straight forward as we could hope. It is not a simple process to store the cells, something which could impact their immune restoring potential and they are highly receptive to their environment, meaning they could be influenced by a rheumatic joint and end up adding to the problem.

Our solution is extracellular vesicles (EVs) – see Figure 2. These are very small particles that all cells use to talk with each other. Therefore, they contain information which reflects the abilities of their parental cells – kind of like biological Royal Mail – see Figure 3. This means that EVs isolated from MSCs could restore balance to the immune system without injecting the cell itself. Moving towards with the goal of making MSC-EVs available to patients, we have been studying them to understand their contents, find ways to increase their production to make treating patients with them feasible and testing whether they would truly be effective in treating RA.

We have now thoroughly characterised our EVs and found promising ways to increase their production, predominantly by combining MSCs from different people, which encourages the cells to 'talk' to each other and increase EV yields. This method of production also seemed to have promising effects when introduced in pre-clinical models of RA, significantly decreasing the swelling and signs of an overactive immune system in the joints - see Figure 4. This is now being translated to patients as we begin to test the effectiveness of MSC EVs when added to the immune cells isolated from patients with RA.

It is our hope that MSC EVs could provide a promising new treatment to the field of RA, that which is effective but lacks the disadvantages of current medications. For example, MSC

EVs could replace the use of steroids during joint flares, which are associated with certain side effects such as high blood pressure or increased infection rates. Nonetheless, it is our hope that this work contributes to the wider knowledge of this field, bringing us one step closer to EV therapies for the future benefit of all patients with immune based disorders.

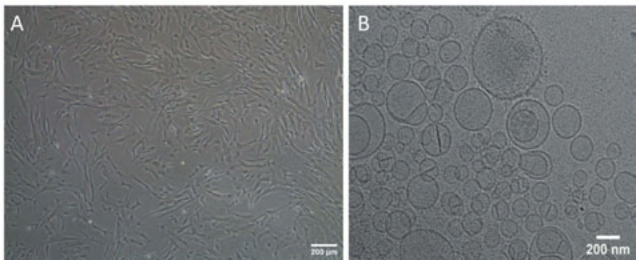


Figure 2. Images of mesenchymal stromal cells in cell culture (A), versus their isolated extracellular vesicles (B).

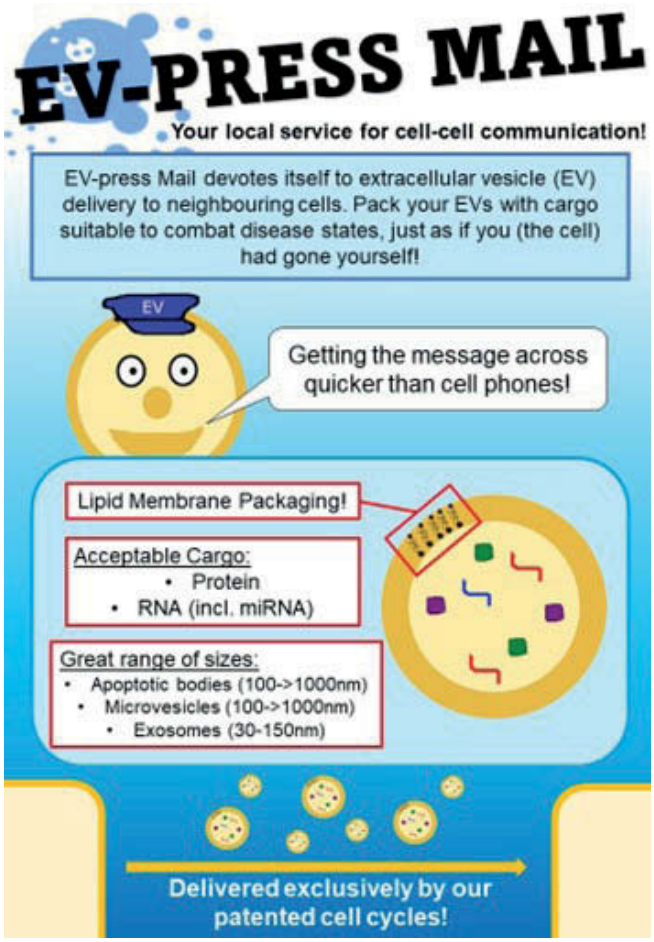


Figure 3. EV-Press Mail: a visual representation of the structure of extracellular vesicles and their ability to act as 'letters' to neighbouring cells, depicted in a format submitted for the purposes of communicating science in an engaging manner.

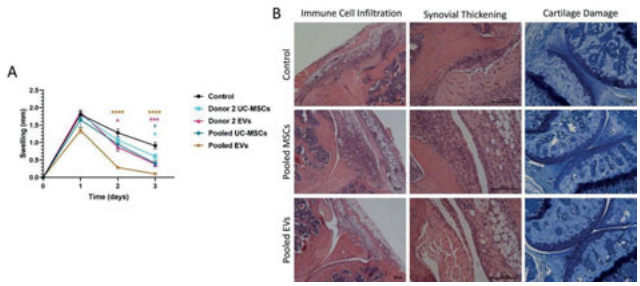


Figure 4. The results of studies involving pre-clinical model of arthritis in which swelling was highly decreased when combining cells from many people and isolating their extracellular vesicles (pooled EVs, orange) to treat the rheumatic joint (A) as well as lessening the amount of immune cells (immune cell infiltration), inappropriate cell growth (synovial thickening) and cartilage damage in the joint (B).

PARIS STUDY: PSORIATIC ARTHRITIS – RESISTANCE TO TNF INHIBITORS STUDY

Anaïs Makos, Dr Jan Herman Kuiper, Dr Roshan Amarasena, Dr Oksana Kehoe

Funded by RJAH Hospital Charitable Fund

Psoriatic Arthritis (PsA) is a disease that can cause pain, stiffness and swelling of the joints and spine. It develops when the immune system attacks the healthy cells of the body, this is what we call inflammatory arthritis. Stiffness, swelling and pain can occur in all joints i.e. hands, wrist, shoulders, hips, knees, ankles, feet and in your spine. In PsA, the cells in blood and joints produce an excess amount of a small protein called Tumor Necrosis Factor (TNF) which is responsible for the swelling and pain of your joints see Figure 5. TNF inhibitors (TNFi) are the first-line biologic treatments able to block this protein to reduce the inflammation. They are prescribed by rheumatologists when conventional treatment does not show any efficiency. However, about 40% of patients do not or only partially respond to TNFi.

The aim of the PARIS study is to find potential biomarkers in blood of patients with PsA to predict response to TNFi. The study also aims to analyse blood from patients with rheumatoid arthritis (RA) to validate, or not, the potential biomarkers in different type of arthritis. A validated biomarker for TNFi response could help clinicians choose a more appropriate treatment in first instance and would improve patients' quality of life faster.

We identified several potential biomarkers, including immune cells and proteins involved in inflammatory responses and immune system activation.

We are particularly interested in a specific cell type, namely T helper cells (Th).

Th1 and Th17 cells are involved in the development of PsA and RA and we demonstrated that high proportions of Th1 cells were associated to a better response to TNFi in patients with PsA Figure 6. These immune cells produce and secrete many inflammatory proteins called pro-inflammatory cytokines. Some of them are overexpressed in patients that respond poorly to TNFi. Some cytokines such as IL-17, IL-12 and IFNγmay be potential biomarkers of response to TNFi and are also involved in psoriasis pathogenesis. Thus, presence of psoriasis might also be a clinical marker of response to TNFi.

The next step is to investigate plasma to find a potential biomarker among hundreds of proteins, and to validate it with ELISA assays. Finally, a large data analysis will be performed, using UK Biobank data to determine how diet, physical activity and comorbidities could be involved in disease severity and lead to the use of biologic drugs such as TNFi.

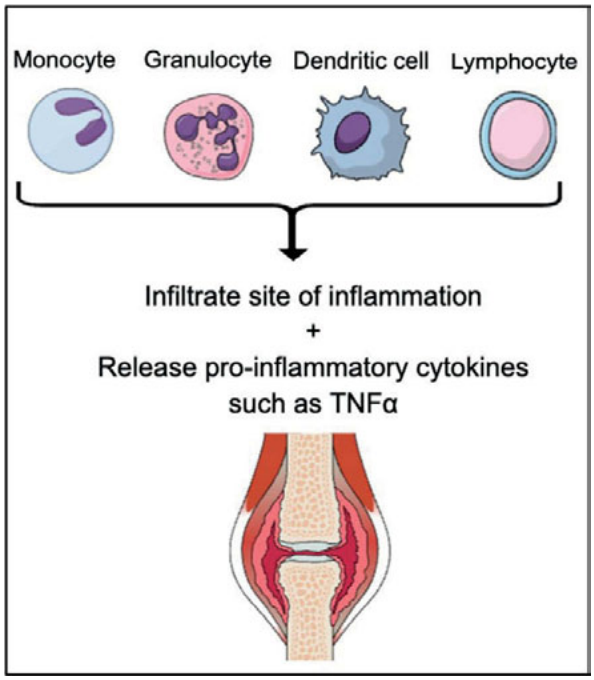


Figure 5. Joint infiltration by immune cells secreting pro-inflammatory cytokines lead to inflammation, swelling and stiffness.

THE POTENTIAL UTILITY OF HUMAN REGULATORY T CELL-DERIVED EXTRACELLULAR VESICLES TO CONTROL INFLAMMATION IN RHEUMATOID ARTHRITIS (VERSUS)

Henry Barrett, Lesley Smyth (University of East London), Dr Charlotte Hulme, Marcelo Andrade De Lima, Professor Aled Clayton (Cardiff University/), Dr Roshan Amarasena and Dr Oksana Kehoe

Funded by the Orthopaedic Institute and Keele University

Rheumatoid arthritis is an autoimmune disorder effecting just under 1% of the population. It causes swelling and inflammation within the joints which leads to joint damage, mobility issues, pain and fatigue. Additionally, 20% of patients are out of work within 2 years of diagnosis leading to further financial and psychological challenges. Although current treatments are available, they aren't always effective and can come with unpleasant side effects. This leads to over 75% of people being taken off first line treatments within a year of starting them.

One promising alternative to traditional treatments is to treat patients with immunosuppressive cells like T regulatory cells (Tregs) see Figure 7. These cells are taken from a patient, cultured and reintroduced back into their system. Recent clinical trials have demonstrated that Treg therapies are safe and cause minimal side effects when used to manage organ transplants and autoimmune disorders like type 1 diabetes. One way these cells keep the immune response in check is by releasing extracellular vesicles (EVs). These small bubble-like structures are made of a cells membrane and contain messenger molecules that calm other immune cells down. As they play such a large role in Treg function, these EVs may be able to treat patients in the same way as Tregs. Although both approaches need more study, Treg-derived EVs may be a cheaper and easier to produce, could be easier to ship and

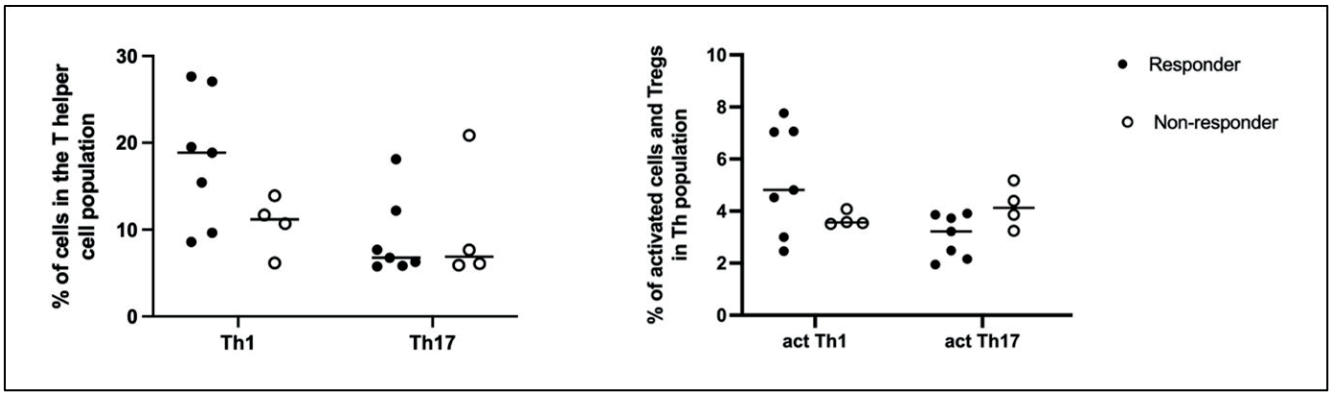


Figure 6. Proportion of Th1, Th17, activated Th1 and activated Th17 cells in blood of patients with PsA. Patients are divided in a responder (n = 7) and non-responder (n = 4) group at 3 months after first injection of TNFi.

store and may be able to treat people other than the original donor. Overall, EVs have the potential to be a more accessible treatment than Treg cell therapy.

At the RJAH, we are comparing Treg-derived EVs taken from healthy volunteers to those taken from people with rheumatoid arthritis. Specifically, we are comparing what messenger molecules are found on these EVs, alongside how well they suppress immune cells. To do this, Tregs will be isolated from blood samples using magnetic cell labelling and then stimulated to produce EVs. Immune cells, specifically T effector cells, will then be treated with varying doses of Treg-derived EVs to see how they change the T effector cells response to inflammatory signals. Finally, both the surface of these Treg-derived EVs and their content will be analysed and compared.

Ultimately, we hope that this project will improve our understanding of how Tregs work, will show if rheumatoid arthritis effects EV composition and suppressive capacity and if EVs from healthy donors would be better treatments than EVs made from a patient's own cells.

Figure 7. Isolated Tregs in culture.

INVESTIGATING THE POTENTIAL OF T CELLS ISOLATED FROM JUVENILE THYMUSES FOR THE DEVELOPMENT OF ALLOGENEIC THERAPIES FOR AUTOIMMUNE CONDITIONS (SELECT)

Dr Oksana Kehoe, Lesley Smyth (University of East London), Professor Aled Clayton (Cardiff University), Mr Rafael R Guerrero (Consultant Congenital Cardiac Surgeon, Alder Hey Children's Hospital, Liverpool), Dr Dan Hawcutt (Alder Hey Children's Hospital, Liverpool)

Funded by the Orthopaedic Institute

One in every 16 of people in the UK live with an autoimmune condition causing them pain, difficulty, lost opportunities in work and in life and in many cases placing them at risk of early death. Autoimmunity occurs when the immune system attacks the body. Regulatory cells (also called Tregs) are cells of immune system which have a role in regulating or suppressing other cells in the immune system. Tregs control the immune response to self and foreign particles and help prevent autoimmune disease. Understanding how Tregs supress cells in the immune system could help improve treatments for people with autoimmune conditions including lupus, type 1 diabetes, Sjögren syndrome, multiple sclerosis and rheumatoid arthritis.

Scientists at the RJAH have a long history of developing novel cell-based therapies for arthritis. To further our studies, we wish to collect discarded human thymuses, routinely removed during paediatric cardiac surgery at Alder Hey Children's NHS Foundation Trust. We intend to study the potential of immune cells derived from thymuses in the treatment of other patients with inflammatory arthritis. Many types of cells and molecules can be derived from tissues removed from patients undergoing cardiac surgery. Some of these tissues have received considerable interest as potential cell sources for cell-based therapies for autoimmune conditions.

The safety of immunotherapy of cells from thymuses (Tregs) have been demonstrated in patients with systemic lupus erythematosus and type 1 diabetes. Results from the first clinical trials demonstrated successful isolation and purification of Tregs, expansion in GMP facilities and re-infusion in patients with improvement in clinical outcomes. Next, this project will explore how the Tregs function. We will add the cells into a dish with immune cells from patients with rheumatoid arthritis. These cells are overactive, which causes the pain, inflammation and swelling in the joints, so we want to see if our Tregs from paediatric thymuses can suppress these immune cells. If these experiments are successful and Tregs contain anti-inflammatory properties, then they have the potential to be applied to many different autoimmune conditions.

This is very exciting project in collaboration with clinicians from Alder Hey Children's Hospital in Liverpool. We are dealing with the ethical approval and recruitment of a Research Assistant. We hope to start this project in late autumn 2023.

THE WOLFSON CENTRE FOR INHERITED NEUROMUSCULAR DISEASE (CIND).

The Clinical Research Team:

Prof Tracey Willis, Dr Richa Kulshrestha, Claire Bassie (specialist nurse), James Jones (DMD HUB advanced nurse practitioner), Nick Emery (senior specialist neuromuscular physiotherapist), Jenny Moustoukas (specialist neuromuscular physiotherapist with respiratory interest), Kate Stracham (specialist neuromuscular physiotherapist), Ellen Thompson (DMD HUB neuromuscular physiotherapist), Kerry Jones (care advisor), Yvette Easthope-Mowatt (Clinical Psychologist), Chloe Perry (DMD HUB trials coordinator), Sarah Clamp (Senior study support officer).

The Laboratory Research Team:

Dr. Heidi Fuller, Prof. Glenn Morris, Prof. Caroline Sewry, Dr Ian Holt, Dr Le Thanh Lam, Dr Sharon Owen, Emily Storey. Affiliate member: Dr Melissa Bowerman (Keele)

Inherited muscle-wasting conditions affect approximately 1 in 1000 people and to date, there are upwards of 50 discrete diseases, each of which is defined by a distinct genetic mutation. Patients commonly present with progressive muscle weakness but the severity and complications vary dramatically between the different diseases.

Research at the Wolfson Centre for Inherited Neuromuscular Disease spans a range of muscle-wasting conditions, with a particular focus on some of the most severe and devastating diseases including muscular dystrophies and the childhood form of motor neuron disease, spinal muscular atrophy.

The clinical research team are actively engaged in several pioneering clinical trials and studies involving patients at RJAH and further afield. The internationally recognised laboratory team, meanwhile, work to find new ways to diagnose and treat inherited neuromuscular diseases.

By designing and developing highly specialised research tools and combining these with the use of cutting edge “omics” technology, their research aims to unravel the complexities of disease mechanisms and identify new targets for therapy development. The laboratory and clinical teams work closely together to promote research and clinical trials as part of clinical practice and access for all. As a group, they are highly committed to training the next generation of scientists and doctors at RJAH and work closely with affiliated Universities at Keele, Manchester and Chester to deliver this.

The Muscle Team continues to hold “Centre of Clinical Excellence” status for paediatric and adult patients, an award given by the Muscular Dystrophy UK (MDUK) in 2019. This is currently undergoing the standard 3-year review audit, delayed due to COVID. The team were also proud to be awarded Duchenne Muscular Dystrophy (DMD) HUB status in November 2019 for DMD clinical trials.

Figure 1. Clinical Research Team photo

The Laboratory Research Team have had a productive year working on active research projects funded by UK-based charities. Beyond the impact of the charity-funded work on advancing research into neuromuscular disorders, grant awards benefit the research group enormously by retaining the best scientists; by facilitating new researchers with valuable skills to join the discipline; by training the next generation of researchers; and by facilitating new transnational research collaborations. The team are particularly grateful to the Orthopaedic Institute for their support of pilot studies within CIND which is a vital step towards gaining proof-of-concept evidence for larger grant applications to external funding organisations.

CLINICAL RESEARCH PROJECTS

The Muscle Team continues to participate in multicentre studies of the natural history of Spinal Muscular Atrophy (SMA REACH project) and the genetics of Duchenne muscular dystrophy (DMD). The Muscle Team continues with its successful ITALFARMACO trial, now in the extension phase after a positive interim analysis, a placebo-controlled trial aiming to reduce fibrosis in dystrophic muscle.

It continues with SMA REACH both for paediatrics and adults and Oswestry was acknowledged recently as one of the four top recruiting sites. They have also fully recruited to the rare

disease project for DMD and have various studies in set up including antisense therapeutics, Sarepta and hydrotherapy trials (all DMD). They were awarded the status of DMD HUB site in 2019 and with this funding for research physio, advanced nurse practitioner and coordinator and all posts have been filled following COVID.

The team have also successfully completed several studies with patients with Facioscapulohumeral Dystrophy (FSHD) in collaboration with both Keele and Liverpool University, including a pilot study of arm cycling in patients with FSHD, a FSHD ultrasound and biomechanics project and more recently a qualitative study, "Best practice conservative non-pharmacological management for patients with FSHD". Prof Willis is also one of the founder members of FSHD UK, bringing together six clinical sites, which aims to harmonise FSHD clinical appointments and enable UK trial readiness for FSHD.

Treatment of SMA has been greatly advanced by availability of gene therapy for severe SMA since mid-2021. Urgent work is now underway via a national group to establish a Newborn Screening programme within the UK. Prof Willis was appointed by NHS England to a fixed-term role on a national gene therapy multidisciplinary team during the set up in the first year in the UK. Nusinersen, an intrathecal treatment, for patients with severe SMA continues now through a Managed Access Agreement (MAA) for both children and adults with SMA type 1,2 and 3 and as of January 2022, Risdiplam, an oral therapy, also became available for these patients as a MAA.

The Clinical Team has been the main hub in collaboration with University Hospital of North Midlands in the West Midlands region for delivering Nusinersen for children in the West Midlands and through Birmingham (UHB) and Salford for the adults who attend Oswestry.

LABORATORY RESEARCH PROJECTS

Spinal muscular atrophy

Without treatment, SMA is the most common genetic cause of death in infants in the UK, and it is estimated that there are approximately 2,000 – 2,500 children and adults living with the condition in the UK. Although recent research has led to breakthroughs in the treatment of SMA type 1, less attention has been given to understanding the molecular pathways involved in the less severe types of SMA.

With funding from Sparks and Great Ormond Street Hospital (GOSH) Children's Charity, a study led by Dr Heidi Fuller and Dr Sharon Owen as postdoctoral research associate, examined the quantities of molecules produced by various cells from patients with differing severities of SMA and compared them to the proteins produced by age-matched control cells. The results of the study showed that the molecular biology of different types of SMA are different. This may have implications for future therapy design, optimisation and efficacy monitoring.

Findings from this study were published in the "Cells" journal, where their article was selected as the "Editor's Choice" and in the "Gene Therapy" journal in collaboration with scientists at Royal Holloway University in London. In addition, their work was shared at national conferences (SMA UK, 2020 and Nerve & Muscle Interest Group, 2022) and internationally (SMA Europe, 2022 – Paris, France; World Muscle Society, 2021 - Online; Neuromuscular Study Group, 2022 – Stresa, Italy) as podium and poster presentations.

Further to the GOSH project, additional financial support was awarded from the Orthopaedic Institute Ltd and Keele University (Faculty Research Fellowship funding, 2022 & 2023) to continue the work and to support the development of additional collaborations both nationally (London, Edinburgh, Salford & Ulster) and internationally (Hannover & Geneva). Current research is examining the possibility of severity-specific biomarkers that may prove useful for monitoring current SMA treatment. In addition, the potential of "old drugs" that have proven to be beneficial in the treatment of other diseases will be examined as possible treatments for adult SMA patients with less severe forms of SMA.

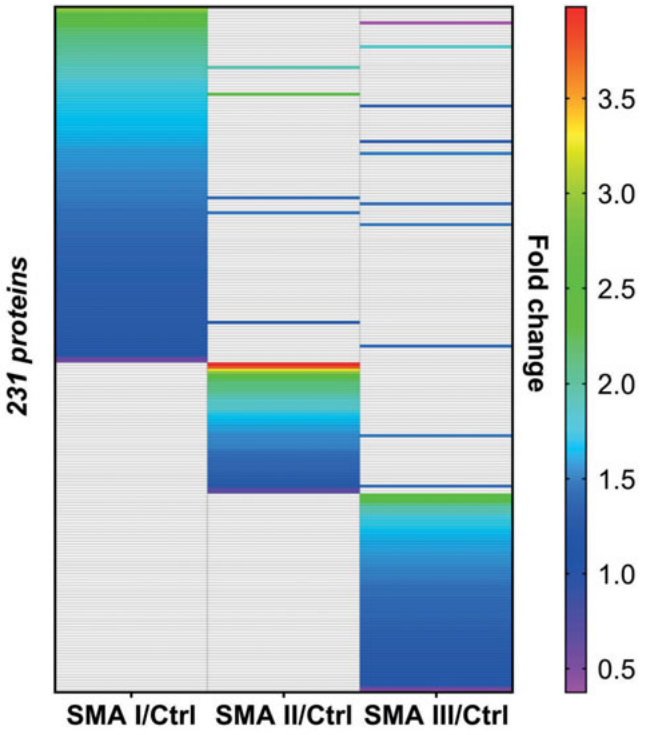


Figure 2. Heat map illustrating the lack of molecular overlap between SMA Type I, II and III

Understanding the effects of glucocorticoids on bone health

Glucocorticoids are prescribed to 1-3% of the UK population for a wide range of conditions

but have harmful side effects including glucocorticoid-induced osteoporosis which is the most frequent cause of osteoporosis in adults under 50 years of age. These negative effects on bone can reduce quality of life and influence survival in certain situations. Vamorolone is a new first-in-human investigational glucocorticoid analogue that may be safer for bone health than routinely used glucocorticoids.

Clinical trials of vamorolone for Duchenne Muscular Dystrophy are underway but it is yet to be approved for routine use or trial for other conditions as fundamental evidence regarding its mechanism of action is lacking.

With generous funding from the Michael Davie Research Foundation, Dr Heidi Fuller and Sharon Owen have begun a project evaluating which proteins are altered in expression following vamorolone treatment of a human bone cell line. These results will be compared to protein changes following treatment with routinely prescribed glucocorticoids. This will help to reveal insights into the mechanisms of drug action, particularly those associated with undesired side-effects and may contribute a case for widening studies of valmorolone to other patient groups at risk of glucocorticoid-induced osteoporosis.

A stable human Schwann cell model of Charcot-Marie-Tooth disease type 1A

Dr Ian Holt and colleagues have been working on a project funded by the Orthopaedic Institute Ltd to make and utilise a human Schwann cell model of Charcot-Marie-Tooth disease type 1A (CMT1A). CMT1A is a hereditary condition affecting the insulating myelin sheath surrounding peripheral nerves which results in muscle weakness and wasting, and loss of sensation. Schwann cells surround the axons in peripheral nerves and produce the myelin sheath. In CMT1A, duplication of the PMP22 gene causes overexpression of peripheral myelin protein 22 (PMP22) in Schwann cells, leading to myelin sheath defects and nerve damage and loss. Therapy development is hindered by limited insights into the molecular pathways involved in PMP22 accumulation and clearance, and by limitations of current disease models, including high cost, time, and variability.

To overcome the limitations of current disease models, human immortalised Schwann cells were engineered with plasmid vectors, to overexpress PMP22. The overexpressed fusion proteins contained a Green Fluorescent Protein (GFP) tag for visual identification and a promiscuous biotin ligase (BioID2) tag for proximity-dependent biotinylation. Control cells, expressing the two tags without PMP22 were also produced. Schwann cells were selected and cloned and the resultant cell lines validated to confirm that they were expressing the appropriate recombinant proteins. Control cells often had a smooth and regular appearance (as seen with non-transfected Schwann cells), whereas those cells overexpressing PMP22 had a spiky irregular appearance. Overexpressed PMP22 exhibited a punctate appearance and asymmetric localisation within the cytoplasm, suggestive of a role in Schwann cell polarity Figure 3.

This work was presented at the Neuromuscular Study Group conference, Italy (2022), for which travel funding was awarded for the work being ranked among the top 15% of all submissions.

The BioID2 tag was used to label and enrich any proteins within close proximity that were interacting with the overexpressed PMP22, including low affinity and transient interactions. The identity of these interacting partners was revealed by proteomic analysis.

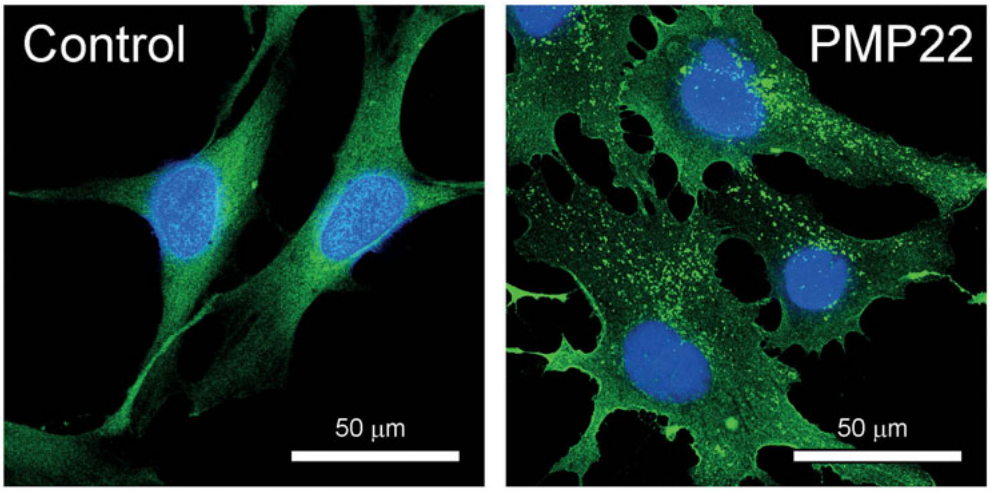


Figure 3. Control Schwann cells (left) were smooth and regular whereas those overexpressing PMP22 (right) were spiky and irregular with aggregations within the cytoplasm

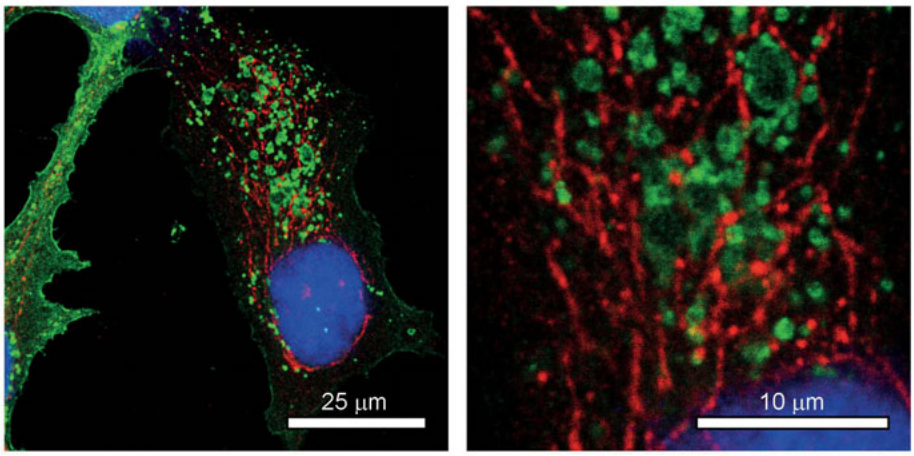


Figure 4. Close association between ESYT1 (red), which is a component of the endoplasmic reticulum membrane and overexpressed PMP22 (green). Low power (left) and high power (right) images

Several hundred proteins were identified in proximity of PMP22. Some of the proteins and pathways represent potential therapeutic targets for CMT1A by promoting degradation and enhanced trafficking of PMP22. The proteins were associated with several enriched molecular pathways including paxillin signalling which is responsible for regulating cell shape, motility and spreading and neuregulin signalling which promotes Schwann cell expansion, survival and myelination. One of the PMP22-interacting proteins was Extended Synaptotagmin-like Protein 1 (ESYT1), which is a component of the endoplasmic reticulum membrane. Microscopic analysis revealed close association between ESYT1 and overexpressed PMP22 Figure 4.

This new cell model of CMT1A will be used to generate insights into the pathological mechanisms associated with CMT1A, to identify targets for therapy design and to screen compounds to evaluate their therapeutic potential, with the aim of reducing the aberrant accumulation of PMP22 in CMT1A.

Lamin-A related congenital muscular dystrophy

LMNA-related congenital muscular dystrophy (L-CMD) is a rare disorder predominantly causing muscle weakness and wasting, which over time, leads to development of dysphagia and life-threating respiratory insufficiency and sometimes cardiac arrhythmias. There are no pharmacological therapies for L-CMD and treatment focuses on managing symptoms of the condition. L-CMD is caused by mutations in LMNA, a gene encoding the nuclear lamina component lamin A/C. Many mechanisms downstream of LMNA mutations in L-CMD remain elusive, making the identification of non-genetic therapeutic targets difficult. During her PhD studies supported by Keele University and the Orthopaedic Institute, Emily Storey has focused on identifying conserved cellular and molecular defects across muscle cells from L-CMD patients, each harbouring different genetic mutations in the LMNA gene. The research has so far identified numerous defects that are common to each of the cell lines. It has also offered insight into mechanisms which may underlie the pathophysiology of L-CMD and has highlighted potential targets for future therapy design studies.

This work has been published in the journal “Cells” and was presented at a range of conferences including Cure CMD, Tennessee (2022) and The World Muscle Society, Canada (2022), where it was selected as a poster highlight from having scored among the top 4% of submissions.

The MDA Monoclonal Antibody Resource

CIND continues to house and run the “Monoclonal Antibody Resource”, originally established with over one million dollars of support from the Muscular Dystrophy Association (USA). Among its successes are collaborations with Sarepta Therapeutics USA and REGENEXBIO Inc USA in the development of novel, FDA-approved treatments for DMD and contributions to the US “SMA Project” which has resulted in new and effective treatments for severe SMA in the UK.

This Resource has produced over 500 new monoclonal antibodies, supplying them around the world (including to the USA, Europe and Japan) for muscle disease research and is also a source of external income. The antibodies are made available to researchers either directly from CIND, from the Iowa Hybridoma Bank (DSHB) and from companies such as Sigma, Millipore and Santa Cruz.

Over the last year, the Resource has sent out a total of 291 units of antibodies across the world to 24 separate researchers. Whilst the majority of these were charged for, some were supplied without cost on a collaborative basis. Direct antibody sales during this period generated £18,600 income, alongside £25,000 income from royalties received from companies that have licensed some of the antibodies, all of which was returned to the Orthopaedic Institute Ltd to support the continued development of the Resource and aligned research.

ORTHOTIC RESEARCH AND LOCOMOTOR ASSESSMENT UNIT (ORLAU).

CLINICAL AND EDUCATIONAL ACTIVITY

ORLAU's clinical services are as busy as ever, with a wide range of patients coming through the doors. Our movement analysis service provides detailed assessment of patients' movement problems, whilst our rehabilitation engineering and orthotic teams prescribe and manufacture medical devices to help them move more easily. Many patients come to ORLAU after having exhausted options elsewhere. Our specialist facilities and expert staff allow us to come up with new and innovative solutions to many complex mobility problems, whether that be for a child with cerebral palsy or an adult who has had a stroke.

During the pandemic years we continued to provide training for clinical scientists specialising in rehabilitation engineering and for physiotherapy students on placement. Other training on site was, however, put on hold to reduce the risk of infection. We are delighted to say that face to face teaching is back, with ORLAU staff contributing to the Orthopaedic Institute's popular Basic Science Course in May 2023 and also running our own Gait Analysis course the following month.

MOVEMENT ANALYSIS

During the pandemic many research projects were paused. As life has returned to normal, we are pleased to report that research is flourishing once again. We have run four large, funded projects, two of which are on-going, and we continue to support students in their research endeavours.

We have previously reported progress on our EPSRC funded project 'Personalised approach to restoration of arm function in people with high-level tetraplegia'. This project aims to use functional electrical stimulation, in combination with an arm support, to help patients who have had a spinal injury to use their arms for functional tasks. The team have successfully configured a stimulator to be activated by EMG signals from the muscles a patient can still use to control stimulation to muscles that are paralysed. The aim is for the system to be adapted to suit the requirements for each individual patient and has now been tested on two patients with further testing planned.

In late 2019 we were awarded funding from the charity Action Medical Research for a project entitled 'Exploration of the role of subtalar joint morphology in the development of foot deformity in cerebral palsy'. The subtalar joint allows the foot to move from side to side and abnormal postures are thought to contribute to the problems children with cerebral palsy experience with their feet. Walking is made more challenging for these children due to deformity and pain. Researcher Dr Erik Meilak has been collecting data in the gait laboratory, along with MRI and CT images of children's feet to build and analyse computer models. The work is leading to exciting new insights into why children's feet deform and Dr Meilak's methods are suitable for application to many different foot and ankle pathologies. He will present his work to date at the conference of the European Society for Movement Analysis in Adults and Children (ESMAC) in Athens in September.

Dr Fraser Philp (from Liverpool University) will also be presenting the results of his research in Athens. He holds two grants, one from the Private Physiotherapy Educational Foundation and the other from the Orthopaedic Institute. Dr Philp is interested in the biomechanics of shoulder instability and he has been measuring subjects with and without instability in the ORLAU gait laboratory. His patient groups include children whose shoulders tend to dislocate or sublux and adults with Facioscapulohumeral Muscular Dystrophy (FSHD), a condition also associated with shoulder problems. Dr Philp has conducted a biomechanical analysis of shoulder motion, with Martin Seyes, a PhD student from Keele University, providing musculoskeletal modelling.

ORLAU's gait laboratory team are active members of the Clinical Movement Analysis Society of the UK and Ireland (CMAS). A small grant from the society has enabled researchers Dr Jo Reeves (Exeter University) and Dr Hannah Shepherd (Liverpool Hope University), along with Dr Caroline Stewart from ORLAU to explore how laboratories across the UK measure muscle activity during gait analysis and how this information is used. This work will also be presented at ESMAC, along with the MSc research of clinical scientist trainee Tim Arthur who has designed a new method for improving the measurement of the walking patterns of amputees.

In February 2023, PhD student Mohammad Alshehab successfully defended his PhD entitled 'The potential for compression garments to influence upper limb', work carried out with the upper limb team in ORLAU, including Mr Rob Freeman, Dr Neil Postans and Mrs Sarah Jarvis. We hope that Martin Seyres and Shallum Sardar will submit their theses later this year. Shallum has been studying toe walking in

cerebral palsy and the effects of treatment with botulinum toxin and plaster casts. Alice Faux-Nightingale successfully defended her MPhil in 2021. Her research was impacted by the pandemic leading to her changing her project and researching the experience of staff in the hospital living and working through the early stages of the pandemic. This research was published in the prestigious Sociology of Health and Illness journal in 2022 and a further paper is being prepared which will focus on the famous hospital corridor.

REHABILITATION ENGINEERING

ORLAU's rehabilitation engineering team has received substantial capital funding this year, allowing us to replace our aging lathe and pillar drill with new machines. These acquisitions will result in increased capacity and precision in manufacturing, and they will significantly improve staff safety. Furthermore, we have acquired a second fused deposition modelling 3D printer to explore and test prototype ideas, leading to patient-centred solutions. This advanced printer operates at higher temperatures, enabling the use of alternative materials in our manufacturing processes.

To complement the printer's capabilities, we have also purchased a handheld 3D scanner. This scanner will enable us to capture highly accurate measurements of complex individual patients, particularly those in need of orthotic extensions to existing devices, where conventional measurement techniques can be challenging.

These scanned measurements can be directly imported into our CAD software, facilitating the development of precise and efficient solutions based on a 3D model of the patient and device.

Our Rehab Engineering team remains committed to offering tailored, engineered solutions to a diverse range of patients. We have successfully undertaken unique adaptations to existing patient devices and have developed innovative solutions that comply with current medical device regulations.

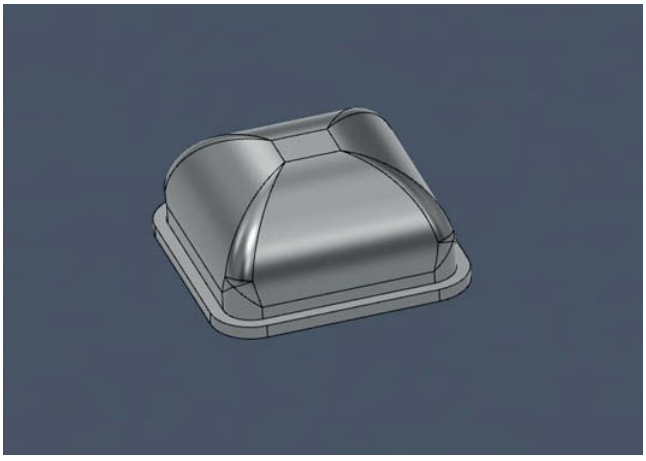
We have purchased a new 3D printer this year, one that can print a wider range of materials. One of those materials is TPU (thermoplastic polyurethane), which exhibits flexible, rubber like properties that we hope to incorporate into some patient-based developments. The images below show a CAD model of a flexible pad and the corresponding 3D printed component, highlighting how a thumb or finger can be depressed into it. This material has the potential to be used for custom cushioning or in devices where flexible parts would be an advantage.

As we navigate the uncertainties posed by the UK's post-Brexit medical device regulations, our department's continued accreditation against ISO 9001 & ISO 13485 places us in a favourable position to adapt to future challenges.

The team is currently working on an exciting redevelopment of the ORLAU Standing Frame. The aim is to replace the existing device in the next few years. The new frame is expected to offer significant benefits, such as improved postural management, easier adjustments to reduce clinic time and enhanced features for convenient device transportation and storage.

Additionally, the team is in talks with a trusted partner about commercialising the mechanical components of the ORLAU ankle Contracture Correction Device. If all goes well, it should be available throughout the UK by the end of 2023.

In conclusion ORLAU continues to be a busy clinical centre, with lots of complex patients coming through the doors every day. Our education and research projects are fundamental to our specialist services. We need to develop innovative new treatments and assessment tools. We are also keen to disseminate our knowledge to others in the field. In both of these areas support from the Orthopaedic Institute is invaluable.



CARTILAGE RESEARCH GROUP

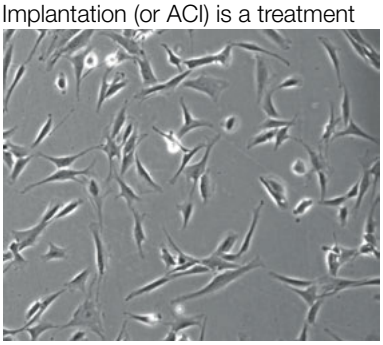
AN EVALUATION OF THE SAFETY AND EFFICACY OF AUTOLOGOUS STEM CELLS, CHONDROCYTES OR THE TWO (ASCOT) FOR TREATING KNEE CARTILAGE DEFECTS

Clinical Delivery and Analysis: *The late Professor James Richardson, Peter Gallacher, Paul Jermin, Martyn Snow, Bernhard Tins, Paul Harrison, Barbara Linklater-Jones, Jo Wales, Naomi Dugard, Jan Herman Kuiper*

Scientific Investigations: *Karina Wright, Helen McCarthy, Claire Mennan, Charlotte Hulme, Jade Perry, John Garcia, Lauren Tierney, Abi Jones, Sally Roberts*

Funded by the Orthopaedic Institute, Versus Arthritis and the Medical Research Council

Autologous Chondrocyte Implantation (or ACI) is a treatment which has been used clinically at the RJAH Orthopaedic Hospital in Oswestry for about 20 years on a select group of patients who have injured or damaged cartilage in their knees or ankles. Instead of using artificial material or the more usual drugs, such as steroids or pain killers, this technique uses cartilage cells called chondrocytes (see picture insert) that have been prepared from the patient's own cartilage and grown in the RJAH's cell culture facility, OsCell's John Charnley Laboratory. The cultured cells are then implanted back into the damaged area in the joint. If left untreated it is believed that these patients would likely go on to develop osteoarthritis with all the changes that this brings in the different parts of the knee, and many would need a joint replacement eventually.



The ASCOT - Autologous Stem Cells, Chondrocytes or the Two? Clinical Trial is comparing chondrocytes with other types of cells which can be obtained from the patient's bone marrow, called mesenchymal stem or stromal cells (MSCs). These have been used in a similar procedure to ACI in Japan. Stem cells have the potential to develop into many different cell types, including those that form both cartilage and bone. MSCs have now also been shown to produce many other therapeutic factors, some of which seem to reduce inflammation and help reduce pain. The question we want to answer through the ASCOT trial is would these MSCs, either alone or combined with chondrocytes, be better at repairing cartilage and bone defects in the knee joint, compared to chondrocytes alone (the standard ACI treatment).

We have now reached our target recruitment of 114 patients for the ASCOT trial and patients have been randomized into the 3 different treatment 'arms' with the 3 different cell populations (MSCs, cartilage cells or a combination of the two). There are 4 patients who are waiting to receive their cells and we aim to treat these remaining patients later in the year. Progress in the study is shown in Table 1, figures correct as of 18/05/2023.

Target number of patients	114
Subjects Enrolled (randomised)	114
Subjects treated	100
Subjects completed final 15 month follow-up	84

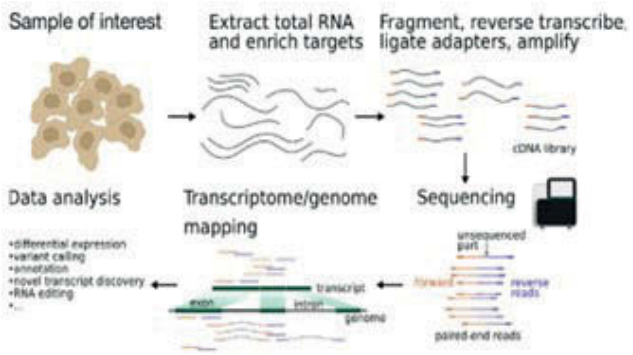
Table 1. Current progress in recruitment and follow-up

SCIENTIFIC ANAYSIS OF THE ASCOT CELL TRANSPLANT – FOCUS ON TRANSCRIPTOMICS AND PROTEOMICS

Abi Jones, Lauren Tierney, Helen McCarthy, Sally Roberts, Charlotte Hulme, Jan Herman Kuiper, Martyn Snow, Dan Tonge, Mandy Peffers (Liverpool University) and Karina Wright

Funded by Versus Arthritis and the Medical Research Council

Using cells obtained from the ASCOT trial, bioinformatics analysis on genes (transcriptomic) and proteins (proteomic) data will be performed. This represents a Versus Arthritis funded PhD fellowship that intends to identify a set of genes and proteins which can differentiate responders and non-responders to treatment, within the different cell therapy groups in the ASCOT trial. By looking at the genes expressed in the chondrocytes and the bone marrow mesenchymal stromal cells (MSCs) the biological activity that is influenced by their transplantation can be determined and we can better understand the mechanism that the cells use to repair the cartilage and restore the function to the joint. The protein content in the same cells helps to confirm that the genes identified are resulting in proteins being made, as well as being easier to measure in a sample than genes in a clinical setting for future applications. We want to ultimately see if there are any proteins which can be used to predict outcome following treatment. The differences in both genes and protein in the ASCOT cells can potentially highlight specific biological pathways involved and expand our knowledge and understanding of the mechanisms responsible for the effects of each of the transplanted cell type(s). Our ultimate aim is to identify a molecular profile(s) that can help predict clinical efficacy.



These analyses will be undertaken using techniques called RNA sequencing for transcriptomic data (see picture insert), and label free proteomics for proteomic data. Both will be performed on cells taken at the time of implantation. These two analyses combined will create a very large dataset which will be interpreted using sophisticated data analysis techniques called bioinformatics. We will be able to identify thousands of genes and proteins from the dataset. The gene and protein signatures found through these analyses will compared with the clinical outcomes of the patients 12+ months on from the implantation surgery to see if there are any genes and/or proteins which can predict clinical outcomes. This can also help to refine or select which cell therapy option is best for clinical use in the future.

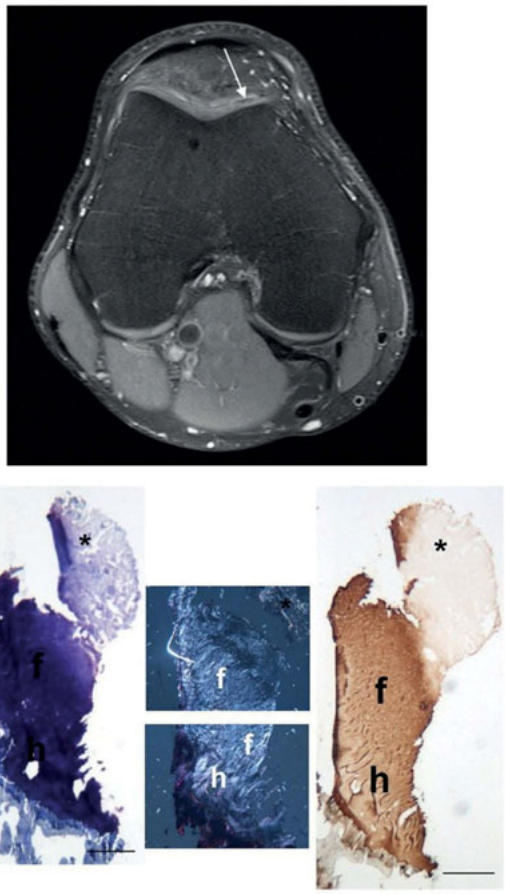
SPONTANEOUS HEALING OF ARTICULAR CARTILAGE (SHARC)

Helen McCarthy, John Garcia, Sally Roberts, Bernhard Tins, Paul Jermin, Peter Gallacher, Oksana Kehoe, Karina Wright, Caroline Stewart, Jan Herman Kuiper

Funded by the Medical Research Council

Osteoarthritis is a painful disease of the joint involving cartilage and bone at the joint. Many factors increase the risk of getting osteoarthritis. Very important among these factors is an injury or a defect of the cartilage. More than 250 years ago, a famous surgeon presented a paper at the Royal Society of London explaining that cartilage, once injured, does not heal. Since those days, doctors and scientists have thought that this is indeed the case. Nobody was therefore surprised that having a cartilage injury or defect is so dangerous when it comes to osteoarthritis.

Research from the past 15 years is now throwing doubt on this old certainty. Researchers who took regular MRI scans of volunteers over time noted that sometimes cartilage defects appear and then disappear. These observations suggest that perhaps cartilage can heal spontaneously or naturally. Over the past five years, we have studied this natural healing process



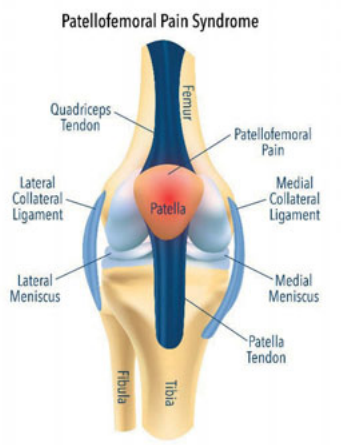
MRI scan of the femur (top) with healed harvest site (white arrow). Bottom row shows a biopsy with proteoglycans (left), collagen structure (middle) and collagen type II (right). The new tissue is a mix of hyaline (normal) cartilage (**h**) and fibrous (not normal) cartilage (**f**).

in our cartilage cell therapy patients. Cell therapy starts with removing a piece of healthy cartilage, from which we grow the cartilage cells. This leaves a defect of around 10-15mm, which fills with new tissue over time. We studied this new tissue after 12 months on MRI scans and by taking a small biopsy from it (see picture insert). The biopsy was used to analyse the structure of the new tissue and what it is made of. What we found showed that although the damaged cartilage did repair itself, it did not return to its normal structure, at least not in one year. For instance, collagens (especially type II) and proteoglycans are very important molecules in cartilage, and we found that their organisation was different in the naturally repaired cartilage compared to normal. This information may help to understand how we can encourage better repair tissue to form.

AN INVESTIGATION INTO PATELLOFEMORAL PAIN: IMPROVING DIAGNOSTICS, PROGNOSTICS AND CELL-BASED THERAPIES

Larissa Rix, Martyn Snow, Jan Herman Kuiper, Andrew Barnett, Karina Wright

Funded by the Orthopaedic Institute

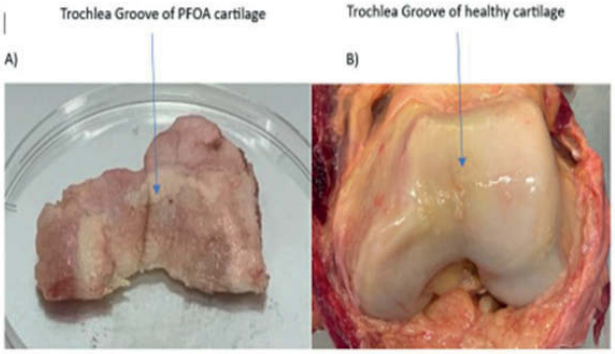


A common disorder seen in orthopaedic clinics is patellofemoral pain (PFP) (see picture insert 1). PFP affects the patellofemoral joint (PFJ), which is made up of the kneecap, known as the patella, and the trochlea groove, of which the patella sits in. PFP typically presents in patients ranging from young and active to sedentary or elderly. Symptoms often occur as pain behind the patella with increasing activity such as squatting, stair climbing or kneeling. With the exact cause of PFP being unknown, diagnosis and treatment are difficult, with most treatment aimed at physiotherapy to target muscle imbalances, or surgical intervention to combat alignment issues. However, when left untreated, PFP can develop into patellofemoral osteoarthritis (PFOA), which occurs when the cartilage breaks down on the surface of the patella, trochlea, or both. At early PFOA, cartilage therapy such as autologous chondrocyte implantation (ACI) can be used to treat the damaged cartilage. Otherwise, at later stage PFOA, the only treatment option is a partial or total knee replacement. Therefore, this PhD project aims to better understand the biology of PFP, and the PFJ, in order to better the diagnosis and treatment of the disorder.

Currently, the main method for assessing cartilage damage is through the use of radiological imaging. Most knee cartilage lesions are diagnosed using magnetic resonance imaging (MRI). However, MRI scans misdiagnose around 20% of cartilage lesions, making it not a highly sensitive tool. Therefore, we assessed how well another method, known as single-photon emission computerised tomography with conventional computer tomography (SPECT/CT), could diagnose cartilage lesions. SPECT/CT works by tracking a radioactive dye to the site of cartilage injury on a SPECT scan and combining the image with a CT scan. This allows the visualisation of the area of cartilage breakdown. Literature around cartilage lesion diagnosis on SPECT/CT was evaluated and we concluded that SPECT/CT may be a useful tool for the detection and localisation of cartilage lesions, particularly in cases where there is an absence of lesions on other imaging tools, or a lack of correspondence with patients' symptoms.

Furthermore, to improve the treatment of cell-based therapies, we have assessed 121 patients who underwent ACI treatment of the PFJ to identify risk factors which influence outcome of ACI. Results showed that factors such as age at time of ACI, the location of the lesion within the PFJ, and the number of cells implanted into the lesion all significantly influenced the outcome of ACI after 1 year.

To further our understanding of PFP and the knee joint of those with PFP, we will undertake regional difference assessment of the knee of patients with PFP undergoing total knee replacement compared to those without PFP in respect to histological landmarks (see picture insert 2), gene assessment and cartilage matrix assessment.



To improve diagnostic and prognostic indicators of PFP, we will assess the synovial fluid of patients treated with ACI in the PFJ to establish biological indicators of the disorder, with the hope to diagnose and treat the disorder at early onset.

THE OSCELL DATABASE

Mike Williams and Karina Wright

Funded by the Orthopaedic Institute

The OsCell database is a clinical research database whose maintenance and enhancement has been funded in large part by the Orthopaedic Institute. The database was originally developed in 2010 but has since undergone significant enhancement and now stores data for more than 15,500 patients. Some of the data goes back to the 1990's, and so there are patients who have more than 20 years of follow up outcome data stored.

Originally it was used to store information for patients who have undergone autologous chondrocyte implantation (ACI) at the RJAH Orthopaedic Hospital. This is a treatment designed to treat chondral/osteochondral defects in the knee, hip, ankle, and elbow. But in recent years it has been enhanced and developed to store information for patients who have undergone a variety of other procedures. It provides a full patient view in that it stores demographic information,

operation history, defect details, cell culture information, biopsy, and histology details, and scoring information for MRI and CT scans. It also enables the storage of a variety of patient outcome scores.

The OsCell database fulfils all the requirements of clinical trials data storage in that it is secure, has restricted access and provides an audit trail allowing the history of each data item to be tracked. It is now the primary data store for a number of clinical trials including ASCOT. Recently, data extracted from the OsCell database has been used by research scientists in a series of high profile publications.

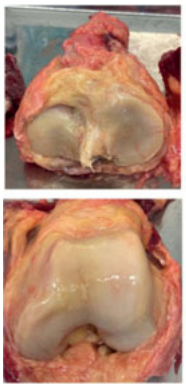
DONATED CARTILAGE STEM CELLS FOR CARTILAGE DEFECT REPAIR – ALLOGENEIC CHONDROPROGENITOR THERAPY PHASE II TRIAL (ACT2)

Jade Perry1,2, Duncan Carroll, Charlotte Hulme, Larissa Rix, Tian Lan, Sally Roberts, Martyn Snow and Karina Wright

Funded by the Medical Research Council

Currently, the most cost-effective treatment for cartilage defects is autologous chondrocyte implantation (ACI) according to the National Institute for Clinical Excellence. ACI involves two surgeries: the first to collect a biopsy of healthy cartilage, which is then used to grow the patients own chondrocytes (cartilage cells) which are then implanted in a second surgery around 4 weeks later. Unfortunately, for many reasons, there has been limited uptake by NHS centres across the UK to adopt the ACI procedure, and the RJAH Orthopaedic Hospital remains one of the few centres to perform this procedure.

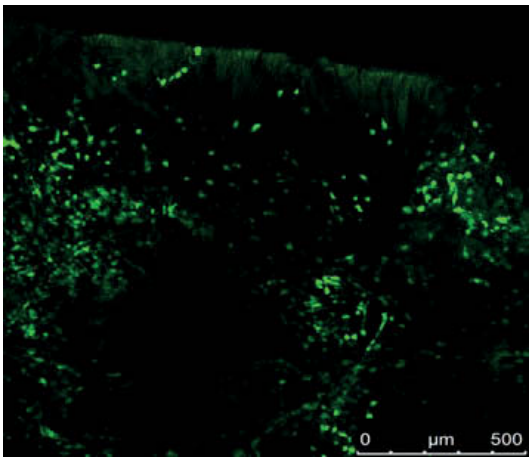
After multiplying chondrocytes in the lab, they can lose their ability to produce cartilage, as they usually do not multiply in the body, so attention has turned to a subpopulation of cells in the cartilage called Chondroprogenitors (CPs). CPs are a stem-like cell, meaning they have some properties similar to stem-cells, such as the ability to multiply and not lose function. The aim of the ACT2 trial is to obtain CPs from cartilage donated through the NHS Blood and Transplant (NHSBT) organ donation service, these will then be multiplied and stored cryogenically until they are needed by patients, where they will be thawed and implanted into the cartilage defect (on a patch made from collagen or hyaluronic acid) in a single surgery. This could result in a higher uptake of ACT over ACI as a single surgery would cost less for the NHS, and multiple patients could be treated from a single donor.



At the RJAH we are optimising the process of harvesting, cryogenically storing and transferring the CPs to the patch (scaffold). This has involved receiving donated cartilage (see picture insert of Prof Snow's dissection) from NHSBT, or US commercial sources (JRF Ortho and RTI Surgical) and processing it to isolate the CPs and testing different variables throughout this process.

In brief, CPs were isolated from full depth human articular cartilage from healthy cadaveric osteochondral allografts from the knee (n=6, aged 24- 42 yrs) and talus (n=6; aged 15- 32 yrs) using selective adhesion to vitronectin. Cell viability across the donors varied from 24—100% and live chondrocyte prep yields varied considerably. Chondroprogenitors isolated from all donors were sterile and were successfully culture expanded. Furthermore, all donors achieved the minimum functionality criteria set for the cells (a measure of their ability to produce cartilage repair tissue components). All chondroprogenitors were immunopositive (>95%) for chondropotency markers, mesenchymal stem cell markers and integrin markers. To date, cryogenic stability testing at 6-months confirmed an acceptable viability value of more than 50% for the 5 grafts to have reached this time point. For the 3 grafts where functionality data is available, the minimum functionality criteria was passed and was above the target value of 90% of pre-cryopreservation values.

In additional work we wanted to assess how the CPs would adhere to the chosen biological scaffolds, and whether the solution they were stored (cryopreservant) in would affect their adherent properties. To do this we seeded 1 cm squares of the Chondro-Gide® collagen scaffold with varying cell numbers in 100 uL of different cryopreservant mixes. Results showed that a cryopreservant with 5% DMSO (an antifreeze



used to stop cells from bursting as they're frozen) performed best for cell attachment, and that a one-centimetre square of scaffold could hold up to 4 million cells.

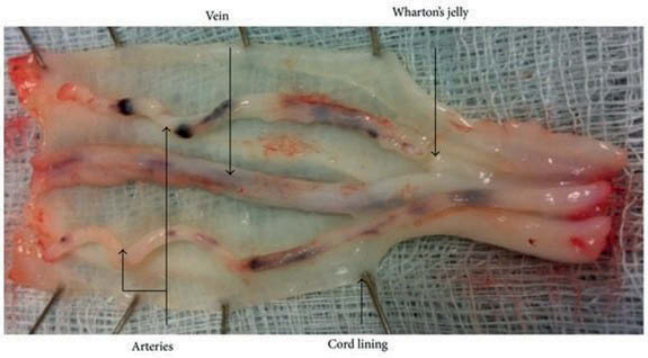
We further assessed cell attachment by isolating seeded scaffolds in their own nutrient media and measured a metabolite of the cells to determine an accurate cell number, as well as visualising the live cells attached (stained green) using confocal microscopy, see picture insert). In the future we will look at differences between two biological scaffolds: Chondro-Gide® (collagen-based) and Hyalofast® (hyaluronan-based), and whether cells perform differently on these scaffolds in terms of cell attachment and cell metabolism.

HUMAN UMBILICAL CORD MESENCHYMAL STROMAL CELLS AS AN INJECTABLE FOR OSTEOARTHRITIS

Claire Mennan, Jade Perry, Karina Wright, Charlotte Hulme, Jamie McDonald, Emma Rand (York), Paul Genever (York), Karin Newall (Cambridge), Fran Henson (Cambridge) Martyn Snow, and Sally Roberts

Funded by the Orthopaedic Institute

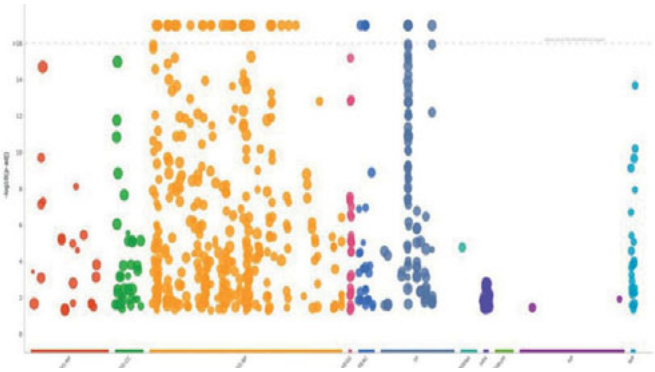
At the RJAH we have been characterising mesenchymal stromal or stem cells (MSCs) from human umbilical cords (UC-MSCs) (see picture below) as a potential therapy for OA for the past 10 years. These are an attractive source of cells for regenerative medicine as they grow well and have anti-inflammatory properties and have been used in humans in many clinical trials around the world. We have been working with collaborators at Cambridge University to see if these cells could delay osteoarthritis (OA) in their early-stage pre-clinical OA model. We used this model to examine the effect of a single injection of human UC-MSCs to see if they can help



repair or regenerate damaged joints and whether the cells reduce joint inflammation and pain. Results showed that there was significantly less OA observed on X-rays when UC-MSCs were used as a treatment. The data from these studies show that UC-MSCs could be an excellent 'off the-shelf' source of cells for the treatment of OA in the clinic, especially for patients with very early OA or an injured knee joint, which is likely to lead to OA in the future.

We have identified a group of patients who we feel would benefit greatly from treatment with these cells, those that have suffered an injury to the Anterior Cruciate Ligament (ACL). However, within this large group of often younger patients, there is a further subgroup for whom UCMSC therapy would be best suited to. The knee joint in these patients often has a lot of inflammation, both from the injury itself and sometimes from the surgery to treat it. Some of these patients have inflammation that never resolves and who may need extra help in the form of a cell therapy. Our studies indicate that an injection of UCMSCs into the joint may reduce inflammation and aid cartilage repair, thereby breaking the vicious cycle of joint damage that can lead to OA. We feel that now is the time to prepare for using these cells in a clinical trial in patients. Changing from growing UCMSCs in a research laboratory, such as we have done, to a 'clean' lab for treating patients requires a lot of additional but often unseen work. For example, some of the reagents which we have used to date come from animals (e.g. cattle) or are not approved for use in humans by the Regulatory Authorities.

To date we have recruited 15 out of our 30 planned ACLR patients as part of our current funding and we have also retested our existing banked samples of synovial fluid from ACI patients for 4 markers indicative of inflammation and cartilage breakdown. Results from this will help us with the ACLR cohort analysis as the ACI group of patients can be studied as an injury model due to the stage 1 and stage 2 procedures involved. We have added Shrewsbury and Telford Hospitals (SaTH) to our existing ethics studies on umbilical cord and to our Orthopaedic Tissues for Research (OTFR) as a sample collection site. This will allow us to collect many more samples for our research, from the maternity unit at Telford and from the acute knee injury clinic at Shrewsbury which will benefit this project and others greatly.



We have successfully used RNA-sequencing analysis in collaboration with York university to assess the potency of cells isolated from different donors' umbilical cords. This analysis revealed large donor variation in response to priming with pro-inflammatory cytokines (to simulate the inflammatory joint environment) and also likely predicted biological pathways

of disease disruption from the UC-MSCs (see picture insert). Priming hUC-MSCs with pro-inflammatory cytokines induced the up-regulation of genes of biological pathways primarily involved in the regulation of immune response, including lymphocyte proliferation and regulation of T-cell cytokine production. This may help to elucidate the mechanisms of action of UC-MSC transplantation for prevention of OA or its early treatment.

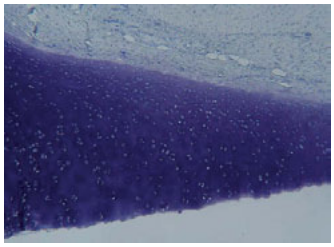
YOUNG HUMAN CHONDROCYTE EXPANSION IN THE QUANTUM® HOLLOW-FIBRE BIOREACTOR

Charlotte Hulme, John Garcia, Claire Mennan, Sally Roberts, Robert Freeman, Nigel Kiely, Derfel Williams and Karina Wright

Funded by the Medical Research Council

The Quantum® bioreactor (see picture insert 1) is an automated hollow fibre system, which has an internal surface area of 2.1m2 (equivalent to 120 T-175 tissue culture flasks), allowing large scale expansion of cells. Our group has used this bioreactor system to grow stem cells from human bone marrow and umbilical cords and has compared the characteristics of these cells to the standard tissue culture plastic technique (TCP), with the aim of using these cells in cell therapies for example, for cartilage repair.

More recently, we have looked at expanding cells from the cartilage, called chondrocytes, in this system. This is because large numbers of chondrocytes will be required to treat cartilage defects, especially is using an allogeneic (donor source) therapy. Also improving cost-effectiveness and batch-to-batch variations of the cells. To date, we have expanded adult chondrocytes from 5 donors who were undergoing total knee replacements. Following 10 million cells being seeded into the bioreactor, an average of 86 million chondrocytes could be harvested in 8 days. These cells retained the same characteristics as matched cells grown using standard TCP conditions, importantly maintaining the same ability to form cartilage.



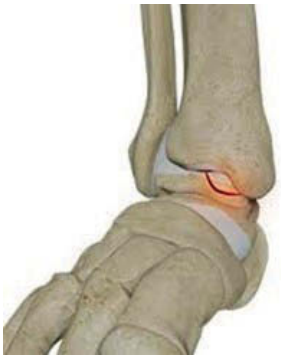
We are currently looking at alternative sources of donor cartilage, which are likely to have capacity to grow well and to repair cartilage. As children, our cells have a better ability to form cartilage, as part of the natural aging/growing process. Therefore, we have identified sources of cartilage from infants aged 4 and under, which are collected with informed patient consent. These include from children born with extra fingers or toes (called polydactyly) that are being surgically removed or from the hip growth plate (called the iliac apophysis) of children being treated for hip misalignment. We have now grown cells from 4 polydactyly (see picture insert 2), and 4 iliac apophysis donors in the Quantum® bioreactor and compared these to standard culture conditions. The greatest number of cells can be yielded from the bioreactor from the polydactyly digits (average 85 million cells). Further characterisation of the cells is being completed to determine which juvenile donor source has the greatest potential for future clinical use.

CELL THERAPY FOR REPAIR OF CARTILAGE DEFECTS IN THE ANKLE – HOW DOES IT WORK AND WHO SHOULD WE TREAT?

Tian Lan, Karina Wright, Nilesh Makwana, Andrew Bing, Charlotte Hulme and Helen McCarthy

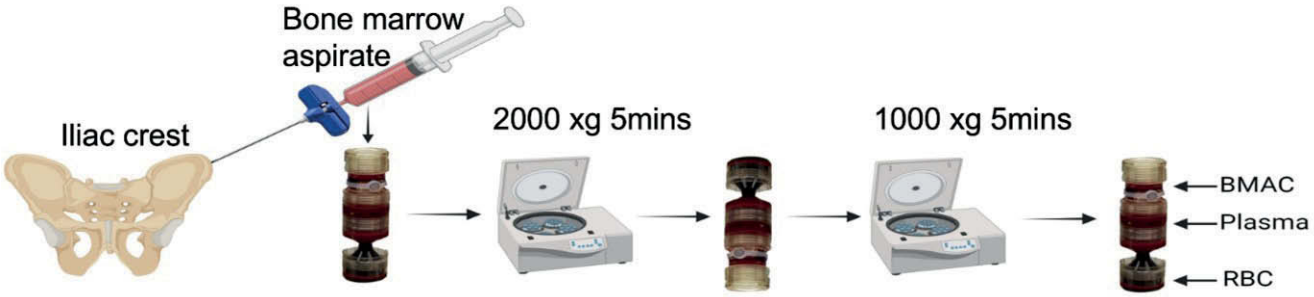
Funded by the Orthopaedic Institute

Cartilage defects often develop in the ankle following traumatic injury, commonly leading to osteoarthritis (OA) if left untreated. Symptomatic patients may experience swelling, locking, catching, prolonged pain and will require surgical interventions. So far, microfracture still remains the “gold standard” for lesions less than 150 mm2. However, advanced cell therapies are required for larger defects and patients who failed microfracture surgery. Autologous chondrocyte implantation (ACI) has been successfully used in treating knee cartilage defects, however, it has not been approved by NICE to use in the ankle.



Ankle surgeons at RJAH have been trialling a new procedure for treating cartilage defects in the ankle (see picture insert 1) using an injection of Bone Marrow Aspirate Concentrate (BMAC) onto the damaged cartilage surface, with promising preliminary results. Briefly, Bone Marrow Aspirate (BMA) is taken from the hip and concentrated using the Complete Cartilage repair (CCR) commercial kit, which uses centrifugation to enrich the therapeutically active components of bone marrow. BMAC is mixed with hyaluronan and fibrin glue, forming a gel which can be injected onto the defect site and holding the BMAC in position (see picture insert 2).

While BMAC has shown positive clinical outcomes, we want to determine which components are therapeutic and which group of people will potentially benefit from this treatment.



Enriched mesenchymal stem cells (MSCs) in BMAC are proposed to be inducers of cartilage regeneration, however, currently there is no point-of-care assessment for BMAC quality; especially regarding the proportion of MSCs within. This part of the study aims to characterise the cellular component of CCR-generated BMAC using a point-of-care device, and to correlate the results with patient clinical outcomes to identify potential therapeutic components in the BMAC.

Donor-matched bone marrow aspirate (BMA), BMAC and BMAC mixed with thrombin (B+T – the CCR treatment) were obtained from consented patients undergoing surgery with CCR. Total nucleated cells (TNC), red blood cell (RBC) and platelet (PLT) counts were measured using a Horiba Micros ES60 haematology analyser, and the proportion of MSCs in BMA, BMAC and B+T were assessed. Our results show BMAC preparations were highly variable in terms of cellular components. Mixing BMAC and thrombin however, as described in the CCR protocol, resulted in a dramatic reduction in TNCs, PLTs and MSCs.

The next stage of this Orthopaedic Institute funded PhD study will be to use a multiplex assay to investigate the cytokine and growth factors in the BMAC plasma layer, and to try to find the active components for BMAC therapy. At the same time, we are also optimising an in vitro co-culture system to mimic the CCR surgical procedure, to investigate which part of the CCR procedure (BMAC or hyaluronan and fibrin glue or both of them) is the active component for cartilage regeneration.

STRATIFICATION OF ORTHOPAEDIC PATIENTS USING BIOMARKERS

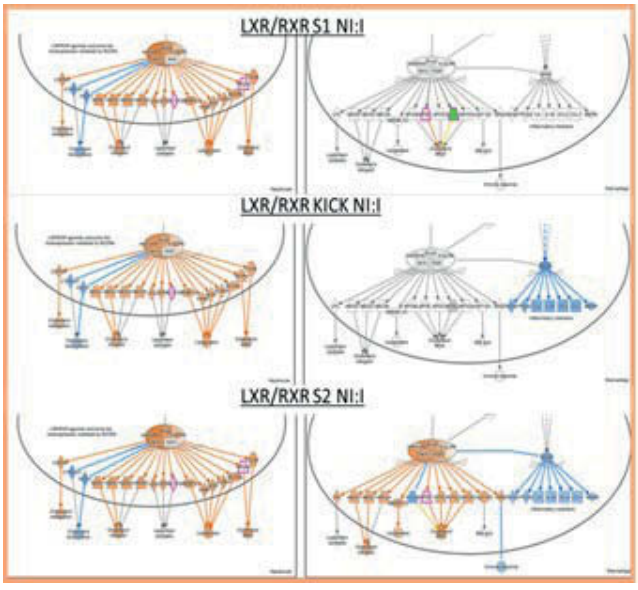
Charlotte Hulme, Jamie McDonald, Claire Mennan, Larissa Rix, Jan Herman Kuiper, Martyn Snow, Pete Gallacher, Paul Jermin and Karina Wright

Funded by the Orthopaedic Institute, Versus Arthritis and the Medical Research Council

In all orthopaedic treatments there is a subset of patients who do not respond as well as others. We want to try and make sure patients are treated with the right surgery, first time to prevent them undergoing surgical procedures that aren't effective and to save NHS costs. We aim to identify molecules in the blood and biological fluids called 'biomarkers' which have different levels in patients who improve compared to those who do not respond. We have been looking to identify biomarkers in the blood or knee joint fluid (which bathes the joint) which can help us to predict a patient's likely response to microfracture, osteotomy, Anterior Cruciate Ligament (ACL) repair and Autologous Chondrocyte Implantation (ACI).

For patients treated with ACI, we have identified a protein called cartilage acidic protein-1, which is measurable in the blood and has strong potential to differentiate between people who are likely to benefit or not from this surgical intervention. This is particularly beneficial for patients because blood samples could be taken in the pre-operative setting and then blood tests used to determine whether or not someone has high levels of cartilage acidic protein-1. Therefore helping in the decision as to whether or not they are suited to this treatment. We now need to measure this protein in many more patient samples before we can confirm how useful this biomarker will be.

In recent work, we have looked to compare patients who have been treated with ACI with other patients who have recently sustained a knee injury, e.g. by damaging their ACL. The reason for comparing these two patient groups is because we think there may be similar changes that happen in the knee following the first procedure in ACI (where surgeons remove a healthy piece of cartilage for extraction of cells that are later



used to repair cartilage damage) and in the weeks following an ACL injury. We want to understand if the people who do poorly following ACI or a knee injury have a similar profile. In the future, if we can pick these patients out, we could offer alternative treatments, which are more likely to work for them. To date, we have identified proteins called Periostin, Vasorin and Complement Factor H that have large differences in concentration between people who either improve or do not following ACI or recent knee injury. Going forward, we will perform further experiments to try and better understand why these patients have different joint fluid concentrations of these proteins.

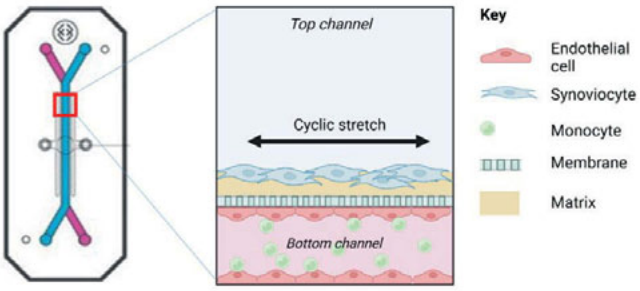
We also analyse the protein information collected using computer software which can assess which pathways in the body are being affected by the treatment or may not be working optimally in some patients. These assessments allow us to identify treatment targets to close the gap between improvers and non-improvers and understand the mechanism for some treatments. We have recently noted that there is a consistent change in the lipid metabolism (see picture insert) in the individuals who do not improve after knee injury or surgery. This is a novel finding, which we hope to develop further in subsequent studies.

ADVANCING HUMAN SYNOVIAL JOINTS-ON-CHIPS

Karina Wright, Martin Knight (QMUL), Timothy Hopkins, Sally Roberts, Charlotte Hulme and Clare Thompson (QMUL).

Funded by the Orthopaedic Institute

There is an unmet need for novel, physiologically relevant models of the human synovial joint for the appraisal of existing treatments, and the screening of new disease-modifying pharmaceuticals, for the treatment of osteoarthritis (OA). Organ-on-a-chip technology can be utilised to generate new in vitro models with a greater capacity to replicate



human physiology in health and disease. Over the course of this proof-of-concept (PoC) study we carried out the initial development and validation of two organ-on-a-chip models of the human synovial joint: 'Vascularised synovium-on-a-chip', and 'Cartilage-synovium on-a-chip'. Both models were developed using the Emulate human emulation system, a commercially available, two-channel, microfluidic chip (see picture insert).

In the vascularised synovium-on-a-chip, healthy human fibroblast-like synoviocytes (hFLS) were cultured in the top channel (to mimic the synovium) and human umbilical vein endothelial cells (HUVECs) in the bottom channel (to mimic a blood vessel), with the two cell populations separated by a flexible, porous membrane. Biomechanical stimulation, in the form of fluid shear and periodic cyclic tensile strain, was applied. The hFLS exhibited characteristic morphology and organisation and deposited characteristic matrix proteins. The addition of an inflammatory stimulus (interleukin-1γ) into the synovium channel was used to mimic synovial inflammation and resulted in the increased secretion of inflammatory and catabolic mediators, as well as the synovial fluid constituent protein, hyaluronan (HA). Enhanced expression of the inflammatory marker, intercellular adhesion molecule-1 (ICAM-1), was observed in the vascular channel, accompanied by increased attachment of circulating monocytes, which were added to the bottom channel under flow. This vascularised human synovium-on-a-chip model recapitulates a number of the functional characteristics of both healthy and inflamed human synovium and can be used to examine human synovial biology and inflammation, identify novel druggable targets and test new therapeutics.

USING GENOMIC SEQUENCING TO DIAGNOSE ORTHOPAEDIC INFECTIONS

Hollie Wilkinson, Helen McCarthy, Karina Wright, Jade Perry and Paul Cool

Funded by the Orthopaedic Institute

The objective of this study is to investigate if genomic sequencing is a useful method to diagnose orthopaedic infections. Traditional methods used to identify the species of bacteria causing orthopaedic infections take considerable time and the results are frequently insufficient for guiding

antibiotic treatment. Current methods include a combination of biochemical markers and microbiological cultures. The aim here is to investigate if genomic sequencing, using a hand-held device (see picture insert) is a faster and more reliable method to identify the species of bacteria causing orthopaedic infections.

Samples of prosthetic fluid are obtained from surgical interventions to treat orthopaedic infections such as revisions or aspirations. Then the DNA is extracted from these samples in the lab and genomic sequencing is performed. This genomic data is analysed to try and identify the bacterial genomes present and therefore, the species of bacteria in the prosthetic fluid sample. The whole process from DNA extraction to output list of bacteria species takes approximately 2 hours, which is considerably faster than microbiological cultures. So far 4 of the samples from confirmed infected patients have been sequenced and the hits for bacteria matching the hospital microbiological culture results have high quality scores associated with them. Further analysis will include looking to identify antibiotic resistance genes in the genomic sequencing data from these clinical samples and see of this information can predict which patients will or will not respond to antibiotic treatment.

The aim is to investigate if genomic sequencing is a faster and more sensitive approach to identifying the species of bacteria causing orthopaedic infections than current methods. This means patients can be diagnosed faster and receive the correct antibiotics sooner, hopefully improving patient outcome from orthopaedic surgery.

THE CHIP STUDY - A LONGITUDINAL COHORT STUDY TO UNDERSTAND CLONAL HAEMOPOIESIS AND IMMUNE MODULATION IN THE HEALTHY AGEING POPULATION AND IN MYELOID NEOPLASMS AND BONE MARROW FAILURE:

Dr Karina Wright, Mr Geraint Thomas, Dr Naomi Dugard, Rebecca Davies, Meryl Owen, Sarah Clamp, Chloe Perry & Lisa Burgess-Collins, supported by the RJAH Arthroplasty surgeons, registrars and anaesthetists.

As we age, genetic alterations (mutations) occur in our blood, which could indicate an increased risk of developing certain diseases, such as heart disease, diabetes, strokes & cancers; including blood cancers. The body's immune system may be able to control and remove these mutated cells, or, in some cases, may make these cells more active and harmful, leading to cancers and other diseases. As similar genetic mutations are observed in people both with and without cancerous blood disorders, the CHIP study aims to understand the events that lead to a higher risk of disease development, leading to improved diagnosis and treatment.

The CHIP study will analyse the blood, bone marrow, somatic DNA and clinical data of patients over the age of 50, both with and without myeloid neoplasms or bone marrow failure. These patients will be followed up over a number of years to assess changes in the blood and medical history. From this data, the study team aim to find out: how often genetic mutations occur in the blood as we age, how changes in the immune system may impact the blood and bone marrow, what these changes mean for both short and long-term health, and the effects of different treatments on the immune system and mutated blood cells.

At RJAH, we participate in the healthy patient cohort and commenced recruitment to the study in September 2021. Bone marrow samples are provided from healthy patients undergoing total hip replacements and to date we have enrolled over 220 patients. Recruitment and follow-up will continue into 2024.

Pictured are members of the team; Meryl Owen, Rebecca Davies, Dr Naomi Dugard, Chloe Perry, Sarah Clamp

BIOMECHANICS LAB

Head of Research: *Dr. Jan Herman Kuiper - Orthopaedic Interventions*

Collaborators: *Mr Andrew Barnett, Dr Kelly Campbell, Ms Taya Chapman, Dr Caroline Dover, Mr Pete Gallacher, Dr Shailesh Naire, Mr Simon Pickard, Dr Nikhil Sharma*

COMPARING TWO METAL PLATES TO CORRECT "MALUNITED" WRIST FRACTURES

Wrist fractures, or to be more precise distal radius fractures, are the commonest fracture in adults, with an incidence rate of over 2000 per million people in a year. Most of these fractures heal well, but in around 10% of cases the bone segments heal in the wrong position, forming a "malunion". Malunion patients have pain, a smaller range of wrist motion and a lower grip strength. Their treatment involves cutting through the malunited fracture, putting the bones in the correct position and fitting a plate holding the bones in the corrected position until the fracture has healed again. A German company started marketing a service allowing surgeons to send them a CT scan of the malunited wrist. The company used the scan to determine the best way to re-position mal-united bone, the best position of the cut, a customized 3D-printed metal plate and a 3D-printed guide for drilling holes in the bones for the screws holding the plate and for guiding the saw to cut the bone. Although the 3D-printed plate will make the surgery easier and hold the bone in an ideal position, it is not clear how good the fixation is compared to clinically proven standard bone plates.

We therefore performed a laboratory study comparing the movements within the fracture gap of an artificial malunited distal radius specimen, which were cut through, repositioned and fixed by either a standard plate or a 3D-printed custom plate (Figure 1). The distal radius specimens were 3-D printed from a CT scan of a patient who had a malunited fracture. We put the specimens with fixated fractures in our materials testing machine and applied forces replicating the average and peak forces measured in wrist fracture patients who do rehabilitation exercises (Figure 2).

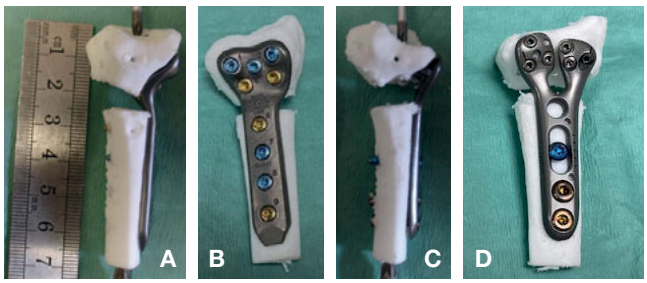


Figure 1. Front and side view of 3D-printed custom fracture fixation plate (a,b) and standard fixation plate (c,d) holding a malunion fracture in the corrected position.

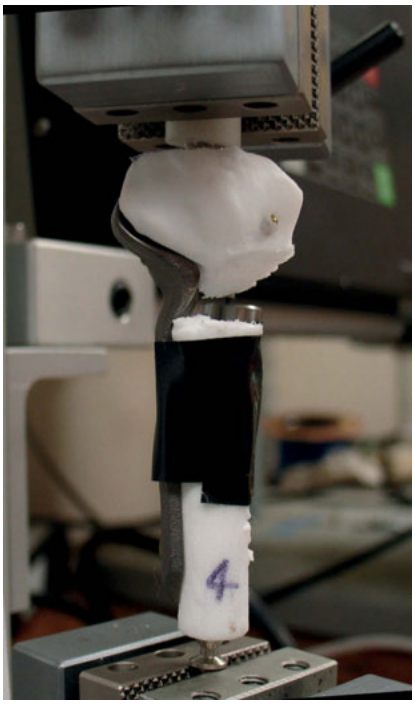


Figure 2. Custom plate clamped in our materials testing machine.

We found that fractures fixated with either plate could withstand 1000 cycles of even the peak measured forces without any permanent change in the bone position. However, the 3-D plate provided a much stiffer fixation, leading to much smaller motions between the bone ends. These smaller motions suggest that patients may be able to start rehabilitating earlier. However, fracture site motion can be beneficial for healing and we don't know if these smaller motions might affect the healing rate.

TREATMENT ALGORITHM FOR CHRONIC KNEECAP INSTABILITY AND THE INFLUENCE OF BODY MASS INDEX (BMI) ON CLINICAL OUTCOMES

Normally, the kneecap (patella) glides through a groove at the end of the femur (thighbone). Patients have a chronic patellar instability if their kneecap repeatedly moves outside the groove completely (dislocation) or partly (subluxation). Patients with a dislocation or subluxation have pain, swelling and difficulties moving the knee.

Patients with chronic kneecap instability can be helped using surgery, but this must be planned carefully because at least three factors can cause chronic instability. First, the patellar groove can be too shallow: if that is the case surgeons can remove some bone to restore it. Second, the tendon that fixes

the kneecap to the tibia might be positioned too far sideways, disturbing kneecap alignment pulling it out of the groove. Surgeons can detach the tendon and reattach it to a better position. Finally, the ligaments that keep the patella inside the groove could be injured and surgeons can reconstruct these.

To help surgeons decide the best combination of surgical interventions, we first developed a system to reliably grade the patellar groove between normal, shallow, flat and convex (Oswestry-Bristol Classification or OBC, Figure 3). Next, we developed a treatment algorithm that used our classification plus a measure of patella alignment to decide the best treatment combination. For instance, only patients with a convex groove (severe abnormality) need their groove deepened, but shallow or flat grooves can be left as they are. We demonstrated that patients treated according to the algorithm had a good clinical outcome with very few complications.

Finally, we studied if patients' body mass index (BMI) had any effect on the result of treatment. The BMI based on a person's weight and height and is often used as a rule-of-thumb to classify someone as underweight, normal weight (BMI=18.5-25), overweight or obese. Patients answered questions about their general quality of life, knee pain and how well their knee functions. Importantly, all patients had a better general quality of life and a better knee after the operation, regardless of BMI. However, we also found that the relations between BMI and general quality of life or knee function had an inverted-J shape (Figure 4). Patients with a BMI around 28 had the best quality of life and those with a BMI around 20-21 had the best knee function after surgery. Studies based on other patient groups also found that people with a BMI around 25, the boundary between normal weight and overweight, report the best general quality of life. However, when it comes to knee function the "best" BMI is in the lower range of normal weight, suggesting that weight loss will benefit all patients but underweight ones.

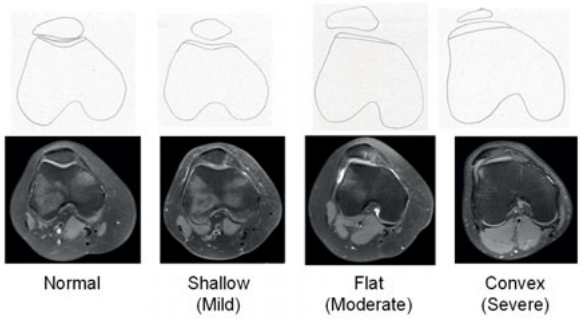


Figure 3. Drawing (top row) and appearance on MRI scan of normal and mildly to severely abnormal patellar groove.

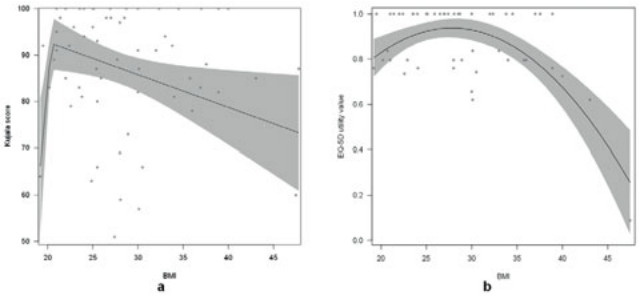


Figure 4. Knee function (a) and general quality of life (b) in relation to body mass index (BMI) after surgery for kneecap instability. Knee function and quality of life have an inverted J-shaped relation with BMI, with the best knee function at BMI=20-21, and best quality of life around BMI=28.

MATHEMATICAL MODEL OF CARTILAGE-BONE DEFECT HEALING

Our hospital has been at the forefront of cell therapy in orthopaedics. We are leading a number of clinical trials in this area and have collected a large amount of clinical data. Cartilage defect patients are treated using Autologous Chondrocyte Implantation, whereby cartilage cells (chondrocytes) are isolated from a small biopsy and expanded in our OsCell cell manufacturing facility, eventually yielding between 1 and 20 million cells. These cartilage cells are implanted into the defect, after which new cartilage grows over time.

Most of our patients who receive cell therapy have defects so deep that they also include the bone underneath the cartilage. Rather than cartilage defects, they are cartilage-bone defects, but the treatment works for them as well. However, instead of a deeper defect filled with only cartilage, these patients' defects fill with new bone and new cartilage, restoring the original tissue. It is amazing that implanting cartilage cells can achieve this result: a bone-cartilage tissue with just the right amount of cartilage on top. The whole process seems very similar to what happens in the growth plate in children and adolescents.

To find out if this might be true and what might control the thickness of the cartilage layer, we formulated a mathematical model based on relatively simple rules that describe several processes that must take place. Our model describes processes such as cell proliferation, migration, differentiation and death, production of cartilage and bone, cartilage calcification, diffusion of nutrients and signalling proteins and the influence of these proteins on the cells, all expressed as mathematical equations. We then used the model to find out

how cartilage-bone defects heal after implanting chondrocytes at the bottom of the defect and what controls the thickness of the cartilage tissue.

Our model predicted that the defect first fills completely with cartilage, taking around 12 months. Next, at the bottom of the defect chondrocytes begin to swell (hypertrophy) and the cartilage around them starts to calcify, after which bone cells were predicted to move in and form new bone. This process was predicted to move as a traveling wave towards the surface of the defect but to stop when around 3mm of cartilage was left at the surface, with a thin layer of calcified cartilage between cartilage and bone. The thickness of the remaining cartilage and the timing of the calcification process depended mainly on specific properties of parathyroid hormone-related protein (PTHrP), a signalling molecule produced by chondrocytes close to the cartilage surface. We think that our model can be helpful in translating between animal models and human patients and in understanding the repair process at a fundamental level.

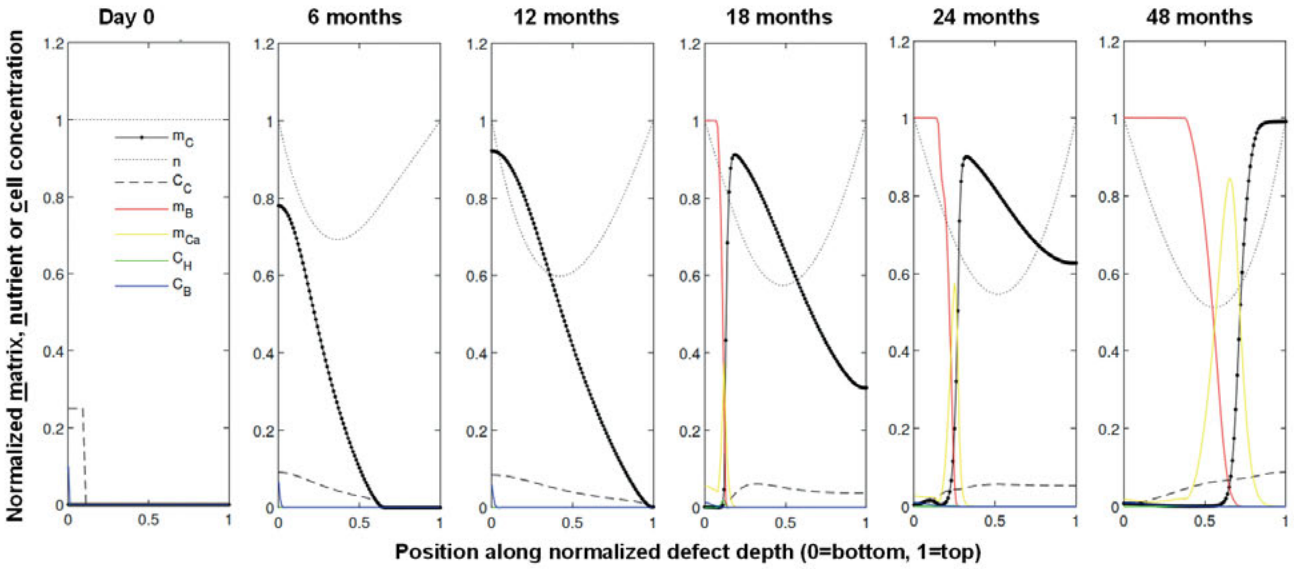


Figure 5. Predicted pattern over time of normalized nutrient (n), matrix (m) and cell (c) density for cartilage/chondrocytes (mc and cc), bone/bone cells (mb and cb), calcified cartilage (mCa) and swollen chondrocytes (cHc) inside a healing bone-cartilage defect after chondrocyte implantation. A density of 1 represents fully mature cartilage and bone or a space filled with 100% cells, and in each panel the horizontal axis represents position along the defect depth from 0 (bottom of the defect) to 1 (top surface of the defect). From 0-12 months cartilage (black diamonds) gradually fills the defect from the bottom up. From 18 months onwards a wave of bone (red line) starts to fill the defect from the bottom up, separated from the cartilage by a thin layer of calcified cartilage (yellow line).

TEPIS

TENNIS ELBOW PLATELET-RICH PLASMA INJECTION STUDY

Platelet-Rich Plasma (PRP) versus Autologous Whole Blood versus Saline Injection in the Treatment of Resistant Tennis Elbow: A Pilot Randomised Controlled Trial

Cormac Kelly, Johanna Wales, Jan Herman Kuiper, Megan Hyne, Leighann Sharp, Julie Lloyd Evans, Charlotte Perkins, Jean Denton, Tessa Rowlands, Claire Nicholas and Deepak Menon

Funded by the Orthopaedic Institute and the British Elbow and Shoulder Society

Tennis Elbow is a common overuse syndrome that causes lateral elbow pain. It is associated with repetitive activity at work and play and is thought to be caused by micro-tears in the tendons of the elbow. Although many cases resolve over a period of 3 months either with or without non-surgical treatments such as rest, exercises and bracing, other treatments such as steroid injections or surgery may be necessary.

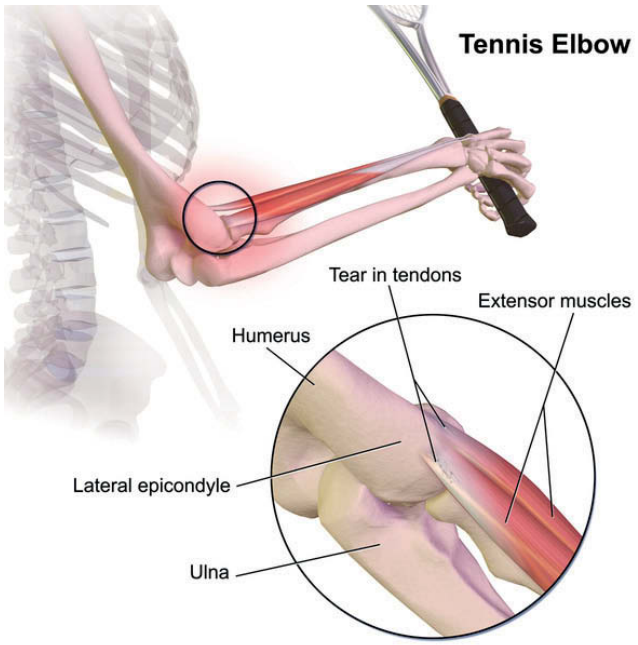
In an autologous blood injection, blood is taken from the patient and re-injected around the affected tendon. Autologous blood injection is thought to promote healing through the action of growth factors on the affected tendon. The injection is given using a technique called needle barbotage that disrupts tendon fibres and is also thought to promote the healing process.

Either whole blood can be injected, or a fragment known as platelet-rich plasma (PRP) can be separated from the red blood cells and then injected. PRP may have a more effective tendon repair potential compared to whole blood, however there is a lack of well-designed studies to support this and there remains variable evidence on whether whole blood or PRP is beneficial to patients with Tennis Elbow.

We chose to carry out a pilot trial to assess the feasibility of designing a larger multi-centre randomised controlled trial to investigate the clinical and cost-effectiveness of autologous whole blood and PRP in the treatment of Tennis Elbow. Patients were allocated to either receiving an injection of autologous whole blood, PRP or saline (placebo).

A target of 10 patients per group (total sample size of 30) was selected in order to inform the number of participants needed in future studies, assess acceptability of study design, and investigate measures that are both clinically relevant and important to patients. Assessments of pain and elbow function were carried out at 6 weeks, 12 weeks, 6 months and 1 year following injection. We also collected information on side effects experienced during the study period.

Patient recruitment and data collection included follow-up data has now completed. 132 patients were screened for eligibility. 29 underwent randomisation, and 25 total patients underwent intervention (7 whole blood, 9 PRP and 8 saline). There were no serious adverse events suggesting treatment is safe. However, recruitment was slower than anticipated, with a large number of patients declining study participation due to PRP injection being available outside of the trial. Collated data is currently in the process of analysis.



SPINAL STUDIES RESEARCH GROUP

BIOMARKER DISCOVERY AND USE IN SPINAL CORD INJURY PATIENTS: IDENTIFYING NEW TREATMENT TARGETS AND MARKERS FOR PREDICTING CLINICAL OUTCOME

Jessica Fisher-Stokes, Sharon Owen, Charlotte Hulme, Mateus Bernardo-Harrington, Joy Chowdhury, Aheed Osman, Srinivasa Budithi, Naveen Kumar, Paul Cool and Karina Wright

Funded by the Orthopaedic Institute and the Midlands Centre for Spinal Injuries

The spinal studies team has continued its work on identifying potential biomarkers in the bloods of patients following a spinal cord injury (SCI) in order to assist in the prediction of clinical outcomes. Recent work looking into the analysis of plasma samples in SCI patients is currently being reviewed for publication. This work identified several biomarkers in the samples that were biologically relevant and have the potential to predict clinical outcomes, many of which have implicated the liver as playing a role in the outcomes of patients post-injury.

Following this, the team is now looking into more targeted analysis of previously identified markers of injury from blood samples. Patient samples are currently being assessed with the use of enzyme linked immunosorbent assays (ELISAs) (see image 1) to determine the concentrations of 2 biomarkers of interest; S100 calcium binding protein B (S100ß), a protein secreted by cells of the central nervous system, and neurofilament light (NF-L), an important structural element of neurons.

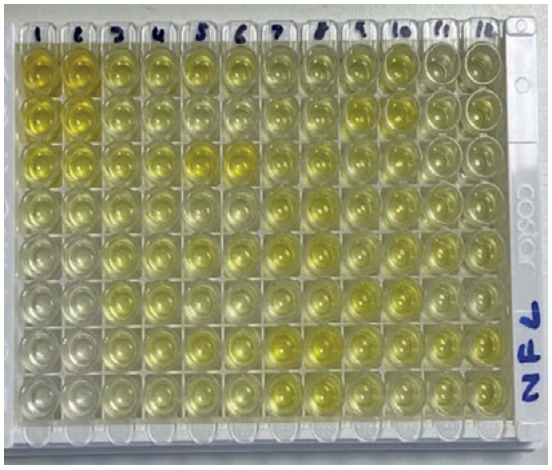


Image 1

Statistical techniques are then being used to determine their potential in predicting neurological outcome in SCI patients. So far, S100ß has shown to be statistically significant in patients between the acute (2-week) and sub-acute (3-month) time point, as well as at the acute time point depending on if they are improvers or non-improvers (see image 2).

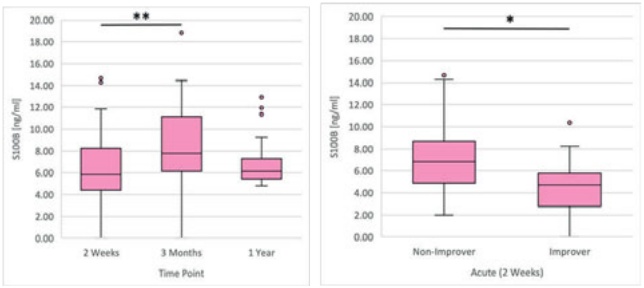


Image 2

We are also beginning to investigate inflammatory markers for their potential as SCI biomarkers. Preliminary work investigated levels in patient serum samples of 9 different inflammatory identified two markers as being of interest, interleukin-1 receptor antagonist (IL-1RA) and interleukin 6 (IL-6), which will also be examined using ELISA techniques. It is hoped that these biomarkers will be able to be used alongside current clinical treatment and help aid rehabilitation decisions to allow patients to achieve the best clinical outcome possible.

The team is also beginning to collect blood samples from SCI patients admitted with pressure sores. These sores are a large problem for patients as they can extend recovery time due to the increased bed rest needed to heal the sores, with spinal injury centers subsequently being unable to free beds for new SCI cases. It is hoped that by investigating markers found in the blood of patients with pressure sores, alongside wound fluids in the future, will help us to understand the occurrence more clearly and often recurrence of the sores, and what factors aid in healing.

Alongside this work, the team is working in collaboration with the University of Glasgow and the NHS Greater Glasgow and Clyde to investigate routine blood scores from their patient population. We previously assessed ~500 patients' blood samples from our hospital to determine which markers that were routinely assessed in the clinical setting would be able to accurately predict patient outcomes. The external dataset we have been given access to from Glasgow will allow us to run the same analysis using their patient data to validate our findings. This will also allow us to see if a different approach to patient management will affect which markers can predict outcomes; our site practices mainly conservative management of patients and surgeries are only recommended when absolutely necessary, whereas other spinal injury units regularly perform surgeries on SCI patients during the initial stage after injury.

THE SPINE MICROBIOME STUDY: ARE BACTERIA IN THE INTERVERTEBRAL DISC INVOLVED IN THE PATHOLOGY OF SOME BACK PAIN PATIENTS?

Jade Perry1,2, Birender Balain1, Bernhard Tins1,2, Sarfraz Ahmad1, Phil Brown1 , Aashish Ghodke1, & Sally Roberts1,2 (Co-I), in conjunction with Profs Frances Williams3 (PI) & Julian Marchesi4 (Co-I) .

1) The Robert Jones and Agnes Hunt Orthopaedic Hospital
2) Keele University
3) King's College, London & Guy's and St Thomas' NHS Foundation Trust
4) Imperial College London

Funded by Versus Arthritis

Low back pain (LBP) is a leading cause of disability globally and commonly believed to be caused by intervertebral disc degeneration (DD), a process which involved the disc as well as other tissues in the spine. Frequent findings are loss of water from the disc, damage to the vertebral bony endplate and in some people, inflammation or fatty infiltration in the adjacent trabecular bone, which is visible on spine magnetic resonance images (MRI), termed 'Modic changes'.

The process is similar to osteoarthritis in the knee, which also involves both bone and cartilage. Like osteoarthritis, DD is more common as we age. To date, the exact sequence of events remains unclear but it has been shown recently that damage to the endplate may happen early in the process and lead to 'leakiness'. This potentially allows the passage of material into the vertebral body such as bacteria, perhaps coming from the large bowel which lies adjacent to the spine, or alternatively which are carried in the bloodstream.

We know that simply brushing your teeth can lead to bacteria in the mouth leaving the gums and circulating in the bloodstream. We aim to determine if bacteria that normally reside in another part of the body somehow reach the disc in question and cause degeneration. The microbial community in our body influences many aspects of health and disease; it may be that it also plays a part in spine health, in particular the health of our discs.

The purpose of this study is to determine (i) whether bacteria are found in any surgically removed discs and if so what type of bacteria are they, (ii) if there is a difference in frequency of bacteria in patients who have Modic changes and those who do not and (iii) if present, where the bacteria may have originated from. Some patients with LBP who are undergoing certain forms of back surgery to remove their discs are asked if they would be prepared to take part in the study. If so, the discs are carefully collected in a sterile container, as well as samples from several other body sites in the patient, including saliva, stool, skin swabs, urine and blood samples, and also swabs from the hands of the surgeon and theatre staff. This is to determine if any bacteria from within the operating theatre may confound the results. All samples are stored in RJAH until the collection is complete (with a target of >50 patients from this hospital).

Patients complete an extensive questionnaire about their clinical status, MRIs are taken and scored and samples eventually sent to King's College and Imperial for analysis of bacterial DNA.

To date at the RJAH we have consented 52 patients, 42 of whom have already undergone disc removal surgery. As this study has an end-point analysis, all scientific investigations remain to be undertaken, apart from an initial pilot of the first 10 patients' samples collected, to ensure that all the transport and other systems were in place. Perhaps by the next annual report there will be some exciting results to report!

PAEDIATRIC SPINAL CORD INJURY AND LONG-TERM SOCIAL OUTCOMES

Dr Richa Kulshrestha, Mr J Chowdhury, Mr A Osman, Mr N Kumar, Mr Budhiti, Mr Jan Kupier, Emma Fosbrook, Julie Ferguson, Charlotte Perkins, Ellen Thompson.

Funded by the Orthopaedic Institute

Spinal cord injuries although uncommon in children, can have a devastating impact on the child, their family and wider society. All areas of the child's life will be impacted for their long remaining lifespan and the effect of their spinal cord injury will be complicated by the impact of their growth and development.

Spinal cord injury patients are vulnerable to a range of complications and will require prolonged specialist care and rehabilitation. The principal goal of such rehabilitation is to achieve a reasonable quality of life.

There is limited research evidence available about the long-term outcomes of spinal cord injury occurring in childhood, especially about quality of life, social activity and participation after reaching adulthood. Thus, the aim of this study is to explore overall quality of life, activity and participation values for adults who have had a spinal cord injury during childhood.

Participants complete a telephone interview and will be compared to matched controls who have had a spinal cord injury during adulthood. The study has almost finished recruiting the childhood injury group and will shortly be moving on to recruiting the adult group.

Achieving a better understanding of the outcomes for childhood onset spinal cord injury is key to understanding the success of treatment pathways and may inform targeting resources more effectively to provide the best possible rehabilitation programs.

EDUCATION

The Orthopaedic Institute course provision continues to be a highly popular programme with a mixture of annually repeated and new courses. All previous courses attracted very flattering feedback from delegates & faculty alike.

We are indebted as always, to the high-quality faculty both from our own hospital and from other centres who continue to be willing to share their time and expertise, passing on their knowledge to the next generation of clinicians.

We are delighted that our courses are back up and running post pandemic. Please see below our courses for 2023. More to be confirmed for 2023 & 2024.

It promises to be an exciting programme of educational opportunities.

Upcoming Courses for 2023

19th & 20th January
Anatomy & Surgical Exposures in Orthopaedics

14th – 19th May
Oswestry Intensive Course in Basic Science in Orthopaedics

28th – 20th June
Oswestry Gait

19th – 20th October
Anatomy & Surgical Exposures in Orthopaedics

10th November
GP Osteoporosis Study Day

15th – 17th November
Spinal Imaging

4th – 6th December
22nd Oswestry Foot & Ankle

11th December
Sports Knee Meeting

Francis Costello Library

LIBRARY & KNOWLEDGE SERVICE

2022 – 2023 saw the Library & Knowledge Service at RJAH continue to rebuild the service following the COVID pandemic which had reduced capacity within the library. During the pandemic the library team of Kenna Blackburn, Yvonne Ankers and Karen James had operated a hybrid model of working with some services delivered remotely.

2022-2023 saw the biggest changes to accessing Library resources at the Trust. April 2022 saw the launch of the HEE funded NHS Knowledge and Library Hub. This provides all HEE and RJAH funded resources in one central place, including the catalogue, e-journals, e-books, Clinical Key, Anatomy TV, BMJ Best Practice, the healthcare databases: Medline, Embase, CINAHL, HMIC and AMED. The hub also houses the Discovery Tool which provides healthcare staff with a user-friendly discovery search tool to streamline searching for all types of high quality trusted evidence-based resources. It was promoted widely in Trust Comms and Social Media and Library staff offered 1-2-1 training, which didn't receive much uptake. The Library also secured sessions at in-service training days at ORLAU, and also in team meetings with Orthotics and Montgomery. We will continue a programme of further training sessions going forward.

Prior to the launch of the Hub, the Library were involved in a lot of behind the scenes work to ensure that all our e-resources would migrate to the platform. In addition, the NHS funded HDAS database was withdrawn. Library staff underwent online training sessions on how to search the native interfaces: Medline, Embase, CINAHL, Amed and HMIC on both the EBSCO and OVID platforms.

In September 2022, RJAH Knowledge and Library Service, along with SATH NHS Library, joined the HEE funded regional Health Libraries Midlands (HeLM) catalogue. HeLM is a new and growing health library consortium incorporating the former NHS BASE Libraries of the West Midlands, Coventry and Warwickshire, Hereford and Worcester, Kettering, Nottinghamshire, Sherwood Forest, Nottingham University, Shrewsbury and Telford, Mid Cheshire and United Lincolnshire health libraries.

The switch to the new catalogue involved nine months of behind the scenes work by the Library Team ensuring all metadata was ready for migration. The catalogue is now integrated into the NHS Knowledge and Library Hub. The new catalogue provides RJAH staff with a wider range of books to borrow.

The Library continues to support our staff and students across the trust by regularly updating our Intranet pages with evidence based articles to support clinical practice.

The Library Team were delighted to receive the Health Heroes award in February 2023 as a result of a nomination for supporting radiography staff in the Trust undertaking their Master's degree.

In April 2022, the Library again secured 160 free books from The Reading Agency and publishers as part of World Book Night. Due to Covid restrictions still being enforced across the Trust at that time, the Library contacted departments directly and shared books with staff and patients across the Trust. The Library shared books with catering & kitchen teams, house-keeping teams, League of Friend Volunteers, Theatres staff, The Movement Centre, Horatio's Garden (to share with MCSI patients), Rheumatology and SOOS. The event is always popular and raises the profile of the Library. The team ensure each year to reach all teams across the Trust, in particular those who are not regular library users.

2022-2023 was a period of change and rebuilding our service, and we look forward to developing our service further in the coming year.

Lis Edwards
Head of Knowledge & Learning

DONATIONS AND FUNDRAISING

Our sincere thanks go out to all those who have supported us throughout the year from grant giving trusts and charities to individual donors. Not forgetting the kind participants and supporters of challenging fundraising events!

It is through your generosity and kindness that the Orthopaedic Institute can continue help fund vital Research and Education here at the Robert Jones & Agnes Hunt Orthopaedic Hospital.

We have had a few fun filled events throughout the year;

The Virtual London Marathon – a team from Lanyon Bowdler Solicitors raised £2,480

An Easter Bake Off – staff collected over £293

The Ultra Marathon – Adam Haythorn ran 50 miles from Manchester to Liverpool donating £830

How you can help...

- **Make a donation:**
  Account Name: Orthopaedic Institute Ltd
  Bank: Barclays
  Sort Code: 20 77 85
  Account No: 53616746
- **Cheques payable to:**
  The Orthopaedic Institute Ltd
- **Organise a fundraising event** with family, friends or colleagues.
- How about a **Team Building Event** at work?
- **Join an organised event** and ask family, friends and colleagues to sponsor you.
- **Donate in Memory of a Loved One.**
- **Leave a Gift in Your Will.**

Donate with JustGiving

www.justgiving.com/orthopaedicinstitute

Don't forget to Gift Aid it!

If you are a UK taxpayer, the Charity can claim an extra 25p from the Inland Revenue per pound donated at no extra cost to you!

If you would like further information please contact:

Debra Alexander
Orthopaedic Institute
Arthritis Research Centre
RJAH Hospital
Oswestry
Shropshire
SY10 7AG

01691 404561

enquiries@orthopaedic-institute.org

www.orthopaedic-institute.org

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ACCOUNTS

The following information shows the Summary Statement of Financial Activities and Balance Sheet, extracted from the audited financial statements for the year ended 31st March 2023.

Statement of Financial Activities including Income And Expenditure Account for the Year Ended 31 March 2023

	Unrestricted funds	Designated funds	Restricted funds	Total	Total
	2023 £	2023 £	2023 £	2023 £	2022 £
Income from:					
Donations, legacies and grants	14,270	-	108,063	122,333	121,282
Charitable activities	96,714	-	-	96,714	139,681
Investments	49,489	-	-	49,489	54,254
Total income	160,473	-	108,063	268,536	315,217
Expenditure on:					
Raising funds	87,042	-	-	87,042	64,366
Charitable activities	270,640	-	58,028	328,668	292,127
Total resources expenses	357,682	-	58,028	415,710	356,493
Net (loss) / gain on investments	(109,151)	-	-	(109,151)	89,303
Net (outgoing) / incoming resources before transfers	(306,360)	-	50,035	(256,325)	48,027
Gross transfers between funds	271,242	(271,242)	-	-	-
Net (expenditure) / income for the year: Net movement in funds	(35,118)	(271,242)	50,035	(256,325)	48,027
Fund balances at 1 April	67,452	2,372,126	1,152,918	3,592,496	3,544,469
Fund balances at 31 March	**32,334**	**2,100,884**	**1,202,953**	**3,336,171**	**3,592,496**

Balance sheet *as at 31 March 2023*

	2023		2022	
	£	£	£	£
Fixed assets				
Tangible assets		1,209,593		1,247,393
Investments		1,515,644		1,682,227
		2,725,237		2,929,620
Current assets				
Debtors	3,112		3,193	
Cash at bank and in hand	708,720		738,487	
	711,832		741,680	
Creditors: amounts falling due within one year	(100,898)		(78,804)	
Net current assets		610,934		662,876
Total assets less current liabilities		3,336,171		3,592,496
Net assets		3,336,171		3,592,496
Income funds				
Restricted funds		1,202,953		1,152,918
Unrestricted funds:				
Designated funds		2,100,884		2,372,126
Unrestricted funds		32,334		67,452
Total unrestricted funds		2,165,904		2,439,578
		3,336,171		3,592,496

Orthopaedic
Institute

Orthopaedic Institute Ltd
Arthritis Research Centre,
RJAH Hospital,
Oswestry, Shropshire SY10 7AG

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