Title of Project: “Assessing peripheral nerve regeneration and immunomodulation in vascularised composite allografts (VCAs) in translational studies using animal models.”

Dear Sir, Madam,

I carried out an 8-week research project at the Vascularised Composite Allograft (VCA) Laboratory at Johns Hopkins University, U.S.A, under the supervision of Dr Gerald Brandacher, who is a pioneer in this field. This offered a unique insight into plastic surgery research at the interface of translational medicine.

I have always been drawn to the continuous innovation of this surgical field with its ability to restore form and function with finesse. Voted the best teaching hospital in America for 21 consecutive years, Johns Hopkins has a rich pedigree in pioneering plastics surgery and translational research including Dr John Staige Davis whose work on Z-plasty and free tissue transfer is still widely used today, and Dr Milton Edgerton who established revolutionary reconstructive techniques during World War 2 and in head and neck cancer surgery.

I had the privilege of working under Dr Andrew Lee who carried out the first bilateral hand transplant in the USA in 2009, and the first above-elbow transplant. Along with Dr Gerald Brandacher, he established one of the only labs in the world that specialises in vascularized composite allotransplantation with emphasis on peripheral nerve regeneration and on immunomodulation through tolerance induction. I was fortunate enough to get an elective offer from this lab, and was looking forward to practicing microsurgery and partaking in research that is pushing the frontiers of modern reconstructive surgery.

Vascularised composite allotransplantation involves the transfer of multiple types of tissue as a single functional unit from donor to recipient, and includes hand and face transplantation, which now represents a viable treatment option for devastating musculoskeletal trauma with extensive tissue loss and has revolutionized the field of reconstructive surgery. Current immunosuppression regimens are necessary to maintain viable grafts, but their extensive side effect profile alters the risk-to-benefit ratio of this non-life-saving procedure, and modern research needs to focus on immune tolerance induction and away from the paradigm of immunosuppressive agents. Advances in peripheral nerve regeneration are important for common nerve lesions, VCA transplantation and improved surgical outcomes in most surgical fields.

Having read through the literature published by the lab, I had been planning on working predominantly on immunology during my time there but after being introduced to the lab Fellows and gaining an insight into their work, I realised that my interests lay more in the peripheral nerve regeneration side of the lab. I contributed to on-going projects and developed skills ranging from cell immunostaining to microsurgical hind limb transplantation. My interests grew and my research ideas were encouraged. Thanks to their hospitality and guidance, I quickly felt like I was part of the team, partaking in animal rounds and scrubbing in for porcine hind limb vascularised composite allotransplants involving many established Attending surgeons who were keen to teach and also learn about Cambridge. I was able to successfully carry out my own rat hind limb transplantation and developed my microsurgical skills under kind supervision from the lab seniors. I helped develop a novel functional assessment protocol for a pioneering VCA model, and wrote a review paper on an agent we were using to encourage peripheral nerve regeneration. Working with mice and rats was a big part of my elective: occasionally I would have to return to the lab in the early hours of the morning to administer immunosuppression medication to the animals in order to prevent limb rejection following transplantation.
One of our key projects involved studying differences in peripheral nerve regeneration and graft rejection between allogeneic and syngeneic rat hind limbs following orthotopic transplantation with some animals administered daily immunosuppression with cyclosporine A (CsA). Orthotopic hind limb transplantation was performed from Brown Norway to Lewis rats, and from Lewis to Lewis rats for allogeneic and syngeneic procedures respectively. Anaesthesia was induced and maintained using isoflurane gas and the right hind limbs of donor and recipient animals including bone, muscle, femoral vessels, sciatic nerve and skin were amputated at the mid-femoral level to produce the vascularised composite graft. Donor limbs were orthotopically transplanted onto recipients with osteosynthesis achieved using cut 18 gauge needles as intramedullary rods, and musculature groups approximated using 6,0 Vicryl suture. Donor and recipient femoral artery and vein were anastomosed microsurgically end-to-end using 10,0 nylon suture, and after blood flow restoration sciatic nerve ends were coaptated with epineural neurorrhaphy using 10,0 nylon suture.

Harvesting the animals involved anaesthesia induction and maintenance followed by skin incision and removal of the sciatic and femoral nerves from both limbs. Following whole animal fixation using intraventricular paraformaldehyde injection with the vascular system as conduits, the soleus, extensor digitorum longus and gastrocnemius muscles from the transplanted limb and the contralateral non-operated host limb were harvested. Following sectioning, muscle was immunohistochemically stained with laminin and analysed using photomicrography to determine cross-sectional area and therefore extent of muscle atrophy and associated successful muscle reinnervation, which was further visualised using alpha-bungarotoxin to stain neuromuscular junctions. Sciatic nerve histomorphometry was used to determine the extent of sciatic nerve neuronal sprouting and reinnervation down the distal (donor) nerve following Wallerian degeneration and was correlated to the extent of muscular reinnervation and bulk. The femoral nerves were immunohistochemically stained for Schwann cell and apoptotic markers to determine the extent of apoptosing Schwann cells in the nerve grafts.

Another key study involved creating an animal model for chronic denervation to create a more realistic simulation of peripheral nerve lesions in humans. Due to the length of neurones in humans and the intrinsically slow rate of neuronal regeneration, microscopic changes occur in denervation target tissue, in particular muscle atrophy. Re-innervation following an extended period of denervation significantly diminished successful regeneration. We showed this using immunohistochemical staining as above, but also using T-cell and macrophage markers in regenerating nerves and target tissue. Nerve histomorphometry and muscle staining supported evidence for this model, which may go on to modify future VCA models.

Many similar techniques were used when assessing peripheral nerve regeneration and muscle atrophy following transplantation with some animals receiving various hormones and drugs to attempt improved outcomes in limb transplantation, with such studies contributing to a greater understanding of the microscopic pathophysiology underlying peripheral nerve regeneration and rejection in vascularised composite allotransplantation.

Such experiments were taken to the next level using the larger porcine model of hind limb transplantation. The donor swine was anaesthetised, intubated and placed supine on the operating table. A groin incision was made and the femoral vascular pedicle identified and isolated. A vascularised paddle of skin was preserved, the tibia and fibula divided at the junction of the upper third to lower two thirds, and the thigh muscles divided at the distal third of the femur, with the femur itself divided 3cm above the knee joint. The recipient animal was prepared in the same way with a subcutaneous abdominal pocket created. The donor femoral vessels were divided and flushed with heparinised saline then microsurgically anastomosed end-to-end on to the host femoral vessels. The well-vascularised skin paddle was exteriorised on the dorsolateral region of the swine for monitoring of immune rejection. To minimise ischemia and anaesthesia times, three surgical teams worked in unison.
Two days prior to hind limb transplantation, selected animals received whole body and thymic irradiation in a linear accelerator for cyto depletion. A daily regimen of antibacterial agents, immunosuppressives and corticosteroids were administered to the pigs initially through central lines and then subcutaneously. This varied between groups of animals with some receiving tacrolimus immunosuppression, and some receiving the biologic CTLA-4 immunoglobulin as co-stimulatory blockade. Daily bloods were taken to determine therapeutic drug concentrations and monitor chimerism development through real-time PCR analysis through identification of SRY in peripheral blood. Postoperative skin biopsies were taken from the donor skin paddles to determine the viability of the allograft by gross inspection and histological analysis. Classification and rejection was then determined histologically using the Banff 2007 criteria.

By liaising with attending surgeons and helping out in operations, learned about operating theatres in America and gained an insight into how surgery differs across the Atlantic; and I was able to contribute to projects on paediatric facial fractures and authored a paper in autologous breast reconstruction. My 8 weeks abroad gave me an insight into academic surgery like I had never experienced it. Despite being at Cambridge University where there is a strong emphasis on academia throughout our medical course, my experiences were predominantly of basic science research, with clinical research only involving retrospective data analyses and auditing. The lab at Hopkins were able to blend together basic science with novel surgical innovation, allowing rapid translation of pioneering ideas in nerve regeneration and immune tolerance to animal models of composite tissue transplantation. I particularly enjoyed the cooperative nature of research at Hopkins. Working closely with labs on campus that were carrying out important research in related fields provided an environment that was particularly conducive to efficiency and problem solving.

Working with visitors from other plastic surgery labs provided an insight into the fierce competitiveness of research at this level, and I was asked not to discuss in detail many of the lab’s novel ideas and innovations outside of the lab.

Talking with other medical students heralded fair warning of the city Johns Hopkins is based in, Baltimore. Immortalised through the TV show, The Wire, Baltimore is indeed a dangerous city and was ranked 9th most dangerous city in the USA by the FBI. Our accommodation was a 10-minute walk from the hospital, and we were quickly advised to “make that a 5 minute run” by many of the doctors we met on our first day.

However, my experience of Baltimore was very positive. I was fortunate enough to have excellent housemates (other medical students doing elective/Summer schemes at Hopkins) and although ambulance sirens, hospital helicopters and gunshots formed a fundamental part of the evening’s ambience, there were plenty of nicer parts of the city and a strong security presence negated any fears of violent crime around the corner. During weekends I would travel to new cities with friends on the East coast and managed to experience a lot of what Boston, New York City, Washington D.C. and Philadelphia had to offer. My elective was an incredible opportunity to experience academic surgery as well as see a new part of the world. It far surpassed any preconceived expectations I had had and I would recommend an elective like this to anybody desiring similar experiences. I had planned out much of my professional future in England but now there are many life-changing decisions to make. Scientific minds never cease questioning the world around them; the beauty of research is that it can provide the answers.

Thank you for considering this retrospective application,

Yours sincerely,

Prateush Singh
Dear Prateush Singh,

I am pleased to inform you that Dr. Gerald Brandacher has accepted you as a visiting medical student for the following elective:
Research elective in Plastic Surgery
for the period of:
June 16, 2014 to August 22, 2014

In order to finalize your acceptance, the following item(s) are needed from you:
HIPAA certification

You will be assessed a registration fee of $300 payable at the time you register in person. In addition, you may be required to sign up for health insurance; if so, this is available at a fee of $269 per month.

In order to access various Johns Hopkins information systems, you will need the following information:
JHED ID: psingh18

As a non-U.S. citizen, you will also require this 9-digit number to use in place of a SSN when logging into the system the first time: 998-60-2415.

Finally, your attention is directed to the following URL that outlines our policies and provides you with more detailed information applicable to your status as a visiting medical student at Johns Hopkins University School of Medicine: http://www.hopkinsmedicine.org/som/vismedstd/vismedaccept.html

Please read through this information thoroughly. If you have any questions concerning your elective after reviewing this information, please reply to this email.

Sincerely,

Mary E. Foy
Associate Dean/Registrar

General information for visiting medical students can be found online at: http://www.hopkinsmedicine.org/som/students/policies/visitors.html