Journal of Anatomy

J. Anat. (2014) **224**, pp228–249 doi: 10.1111/joa.12134

Anatomical Society Summer Meeting 2013: Form and Function in Regenerative Medicine & The Dark Art of Learning Outcomes

Royal College of Surgeons in Ireland, Dublin, July 4th & 5th 2013

FORM AND FUNCTION IN REGENERATIVE MEDICINE ORAL COMMUNICATIONS

S1 Cartilage form and function in health, disease, and regeneration

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Osteoarthritis is a painful and debilitating disease of the joints that is characterized by progressive degeneration of the articular cartilage that lines the joint surfaces. The etiology of osteoarthritis is poorly understood, although it is now well accepted that biomechanical factors play an important role in the onset and progression of this disease. The primary goal of our studies has been to determine the mechanisms by which mechanical loading affects the physiology of our joints. Using a hierarchical approach to span different systems ranging from clinical studies and *in vivo* animal models to studies of tissue, cellular, and subcellular anatomy (form) and biomechanics (function), we have identified specific mechanical signaling pathways that are critically involved in cartilage physiology as well as pathology. These pathways may provide novel pharmacologic targets for the modification of inflammation or cartilage degeneration in osteoarthritis. Additionally, our studies have focused on tissue engineering approaches for repairing cartilage damage with osteoarthritis. Using novel textile processes that allow weaving of biomaterial fibers in three dimensions, we have created functionalized bioactive scaffolds that can recreate many of the complex biomechanical properties and anatomic features of articular cartilage. In combination with a multipotent population of adult stem cells, we have developed a tissue-engineering approach for complete resurfacing of osteoarthritic joint surfaces. Taken together, these studies emphasize the important roles of form and function in the health, disease, and regeneration of the joint.

S2 Tissue engineering scaled-up, anatomically accurate osteochondral constructs for joint resurfacing

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Partial and total joint replacements are the only surgical procedures currently available to treat articular cartilage degeneration associated with diseases such as osteoarthritis. An alternative to this procedure would be to tissue engineer an anatomically accurate osteochondral construct and use it to replace the diseased joint, however the generation of tissues of sufficient functionality and scale to resurface an entire joint remains a significant scientific and engineering challenge. The global objective of this study was to tissue engineer a scaled-up, anatomically shaped, osteochondral construct suitable for partial or total joint resurfacing.

We first sought to determine the combination of cell and scaffold type that could be used to tissue engineer phenotypically stable cartilage overlaying functional bone. The chondral layer of these osteochondral constructs were formed by combining co-cultures of chondrocytes and mesenchymal stem cells (MSCs) in either agarose hydrogels or self-assembled tissues, while the underlying osseous layer was formed by combining bone marrow (BM) derived MSCs in alginate hydrogels. Agarose hydrogels and self-assembled constructs were seeded with the following cell types: Chondrocyte (CC) only, BM-MSC only, fat pad derived MSC (FP-MSC) only, BM-MSC & CC (4:1 ratio), and FP-MSC & CC (4:1 ratio). After 42 days of *in vitro* culture under chondrogenic conditions, self-assembled constructs were found to accumulate more sGAG and collagen than agarose hydrogels when normalized to wet weight. All co-culture groups formed stable cartilage *in vivo* following subcutaneous implantation into nude mice. Bone formed in the osseous layer of all implants *via* endochondral ossification.

In the second phase of the study, anatomically accurate MSC-seeded alginate constucts mimicking the geometry of the medial femorotibial joint were generated from moulds fabricated by rapid prototyping. These constructs were covered by a self-assembled layer of engineered cartilaginous tissue (BM-MSC & CC co-culture). After 6 weeks of *in vitro* culture, the scaled-up constructs were implanted subcutaneously into nude mice. After 8 weeks *in vivo*, a layer of phenotypically stable cartilage remained on the surface of the engineered implant, with evidence of immature bone development in the underlying alginate layer. These findings open up the possibility of a tissue engineered treatment option for osteoarthritis.

S4 Structure and symptom-modifying therapies for discogenic back pain

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Low back pain is the second most frequent cause of doctor visits and is commonly associated with intervertebral disc (IVD) degeneration. There are few minimally invasive treatments available for IVD degeneration. Recapitulation of the processes

that occur during development of the IVD can help inform therapeutic strategies for discogenic pain. The objectives of this work are to review the literature and to present new data that: (i) describe sources of discogenic back pain; (ii) describe current treatments for disc degeneration; and (iii) identify therapeutic candidates that are derived from notochordal cells. Methods involve a combination of literature review and new data generation. Painful IVD degeneration is known to be associated with structural disruption, chronic inflammation, and neurovascular ingrowth, all of which must be addressed to promote function and inhibit painful conditions in the spine. Structural defects promote altered spinal biomechanics and must be repaired. Inflammation of injured spinal structures surrounding the IVD may increase the local inflammatory environment, yet it is unknown if TNFα can be transported into intact IVDs and whether this can be an initiator of the known catabolic shift in degenerated IVDs. In the developing IVD, the notochord patterns all regions of the IVD creating a large avascular and aneural IVD through the secretion of several important ligands (e.g sonic hedgehog, connective tissue growth factor, and chondroitin sulfate). A shift in nucleus pulposus cellular phenotype from predominantly notochordal cells (NC) to predominantly small nucleus pulposus cells is associated with maturation, and the loss of NCs has been speculated to initiate the onset of IVD degeneration in humans. We propose that these developmental ligands have the capacity to influence cell differentiation, promote structural repair and to inhibit the neurovascular ingrowth associated with painful IVD degeneration.

Acknowledgements: This work was funded by NIAMS/NIH Grants (R01 AR051146, R01 AR057397, and R01 AR064157).

S5 Harnessing the form and function of pre-vascularisation and embryological bone formation as novel approaches for bone repair using tissue engineered collagen-based scaffolds

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Bone tissue engineering (BTE) has developed due to a severe lack of adequate alternatives capable of enhancing healing in critical-sized defects. Traditional approaches frequently encounter the major obstacle of insufficient blood supply in the implanted tissue resulting in avascular necrosis and construct failure. We have recently developed a mechanism to engineer, *in vitro*, microvasculature within cell-seeded collagen-glycosaminoglycan (CG) scaffolds to overcome this problem. The *in vivo* response and functionality of this system was assessed in critical-sized rat calvarial defects and compared to cell-free CG scaffolds (control). Quantitative analysis with microCT and histomorphometry demonstrated increased bone formation in the cell-seeded groups compared to controls. M1/M2 immunohistochemistry revealed the potentially beneficial immune response towards these BTE constructs during tissue repair. Appendicular and axial skeletogenesis and most fracture healing occur via endochondral ossification (ECO). Hypertrophic chondrocytes releasing angiogenic factors such as vascular endothelial growth factor (VEGF) as well as an early marker of osteogenesis, alkaline phosphatase, promote vascularisation in a cartilage pre-cursor prior to bone formation. We have developed an *in vitro* model to re-capitulate the process of endochondral bone formation using rat mesenchymal stem cells in the presence of chondrogenic factors (TGF-β3) and our biomimetic collagen-based scaffolds. Cartilage matrix deposition, collagen X and VEGF gene expression and mineralisation show the potential of this system to stimulate angiogenesis and subsequent bone production. *In vivo* assessment of this model to assess the healing of a mid-diaphyseal femoral defect is currently underway.

HRB:PhD2007/2011&Grant No: HRA_POR/2011/27; Science Foundation Ireland; European Research Council (grant agreement no. 239685).

S6 Recreating the endochondral ossification process as a bone regeneration strategy: chondrogenic and vascular priming of mesenchymal stem cells enhances osteogenesis *in vitro*

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Endochondral ossification is a tightly regulated process, which relies on the establishment of a cartilaginous template and vessel invasion prior to bone formation. In a recent study we found that chondrogenic priming of mesenchymal stem cells *in vitro* can increase their potential for osteogenic differentiation and mineralisation, even more so than culturing the cells in osteogenic growth factors alone. Other studies have shown that pre-vascularisation of MSCs with human umbilical vein endothelial cells (HUVECs) can enhance osteogenic differentiation of MSCs. However, the use of MSCs for osteogenesis has not been fully optimised to regenerate bone tissue *in vitro* for clinical applications. Although chondrogenesis and vascularisation are crucial bone formation *in vitro*, no *in vitro* bone regeneration strategy has sought to incorporate both events. The objective of this study is to chondrogenically prime MSCs to form a cartilage template and subsequently pre-vascularise the constructs through co-culture to further enhance the osteogenic potential of MSCs.

Human bone marrow derived MSCs were extracted from bone marrow aspirates of four donors (45, 48, 56, 59 years), expanded, trypsinized and centrifuged to create cell pellets. These pellets were further cultured under the following conditions (i) chondrogenic priming (CP) for 14 days, (ii) CP for 21 days, (iii) CP for 14 days followed by vascular priming (VP), through addition of HUVECs at a ratio of 50:50 per pellet, for 14 days, (iv) CP for 21 days and VP priming for 14 days and (v) osteogenic priming. Biochemical (DNA, Alkaline phosphatase (ALP), Calcium and VEGF), histological (Alcian Blue and Alizarin Red), and immunohistological (CD31) analyses were performed.

Our results show that chondrogenic priming for 14–21 days, prior to being exposed to osteogenic factors, produces a cartilage template. This template provides a suitable platform for HUVECs to attach and proliferate, and leads to a significant reduction in the expression of VEGF. This might indicate that

HUVEC co-culture negates signalling by MSCs for vessel invasion, but further studies are required. Ongoing studies are investigating whether chondrogenic priming followed by vascular priming and mechanical stimulation can serve as an effective approach to regenerate bone tissue.

S8 Mild heat-shock provides an effective stimulatory effective for bone regeneration by osteoprogenitors *in vitro*

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Thermotherapy has been widely used to treat a number of diseases including inflammatory osteoarticular disorders, malignant bone tumors and bone metastasis. A previous study has shown that hyperthermic treatment (42.5–44 °C) of rabbit femur after undergoing surgical trauma, stimulated bone remodelling, trabecular bone formation and increased cortical bone density. However, the use of heat-treatment to enhance osteogenesis by mesenchymal stem cells (MSCs) has not been fully explored to regenerate bone tissue *in vitro* for clinical applications. In this study, we investigate the direct effect of temperature on the osteogenic potential of osteoprogenitors using (i) direct exposure of MSCs and MC3T3-E1's to elevated temperatures and (ii) a MLO-Y4/MSC co-culture and heat treatment technique.

Balb/c MSCs, osteoblast-like MC3T3-E1 and osteocyte-like MLO-Y4 cells were maintained in culture media (α-MEM, 10% FBS, 2% penicillin-streptomycin and 1% L-glutamine) prior to the following experiments: (i) Balb/c MSCs and MC3T3-E1 cells were exposed to pre-heated media at 37° (control), 45°, 47° and 60 °C for 30 s and 1 min on a hot plate, (ii) Balb/c MSC's were cultured with previously heat-treated MLO-Y4s (at 37° (control), 47° and 60 °C for 1 min), which were physically separated using PET membranes with 1 μm pores, 30 min after heat treatment. In both experiments osteogenic differentiation was quantified by monitoring alkaline phosphatase (ALP) activity, calcium deposition and cell number up to 14 days.

The effects of heat-treatment on bone regeneration capacity were dependent on the temperature elevation, the duration of exposure and the phenotype of the cell. After 14 days recovery an increase in ALP production and calcium deposition, indicating osteogenic differentiation, occurs in MSCs and MC3T3-E1 cells exposed to temperature elevations. However the cell population was considerably affected when exposed to temperatures >45 °C for 1 min. Interestingly we found that a co-culture technique, wherein MSCs were co-cultured with heat-treated MLO-Y4s, lead to increased alkaline phosphatase activity and calcium deposition compared to the control, and the population size is maintained. These results indicate that thermally induced cellular responses might stimulate bone regeneration, and might inform future *in vitro* strategies to enhance MSC differentiation and bone regeneration for clinical applications.

S9 Cells meet textiles

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Introduction: The biomechanical properties of the human body are mainly defined by fibre structures, like e.g. collagen bundles, elastic fibres, fibrin fibres, fibrous cartilage, and ligament etc. The aim of tissue engineering is to replace, repair, maintain and/or enhance tissue function. Herefore the classical tissue engineering requires an ideal combination of cellular component, scaffold materials and biomechanical and/or biochemical signals. The material plays a central role with regard to the 3D structure, the cell-to-cell-interaction and the biomechanical properties of the complete construct.

Methods: Textile Engineering offers a multi-scale toolbox for the development of scaffold structures on (i) the molecular level of polymer science and biochemical functionalisation, (ii) the nano/micro-scale level of fibre production (e-spinning, melt-, wet-, dry-spinning) and on a (iii) meso/macro-scale level for the production of 2D and 3D structures by weaving, knitting, braiding etc.

Results: Different textile technologies have been successfully evaluated as single technology or in combination with regard to cardiovascular and pulmonary implants like:

1 Non-wovens as scaffold material for heart valve tissue engineering
2 Warp-knitted structure for textile-reinforcement of biological vascular grafts and stents
3 Braided structures for endobronchial stenting
4 Combination of single-fibre-placement and e-spinning for biomimetic, textile-reinforced heart valve prosthesis

Summary: A variety of textile-based and textile-reinforced cardiovascular and endobronchial implants have been developed in our group. The presentation will give an overview about the different textile technologies and their impact for tissue engineering in general and cardiovascular tissue engineering specifically.

S10 Development of bilayered tubular collagen-elastin scaffolds for vascular tissue engineering

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Cardiovascular disease is the leading cause of death worldwide, annually accounting for 17.3 million deaths which represents 30% of all global deaths. Arterial bypassing, for small diameter vessels (<6 mm), suffers from poor patency rates using synthetic

or autologous grafts due to thrombosis, aneurysm formation, and a compliance mismatch. Thus, the focus of this project is the development of a tissue engineered blood vessel (TEBV) for small diameter vessels.

Elastin is a key component of native vasculature where it is responsible for the elastic recoil of vessels and has been shown to have a role in smooth muscle cell (SMC) behaviour. Thus, in this study, an elastin and collagen tubular composite scaffold was developed with the aim of providing a more natural viscoelastic response and to control SMC activity. An advanced biofabrication technique was developed which enabled the construction of a bilayered tubular construct via a combination of dehydration, freeze-drying and crosslinking. The resulting construct consisted of a highly porous outer layer for SMCs and a dense film layer to inhibit intimal hyperplasia and support endothelialisation. Results were evaluated via mechanical testing, histology, SEM, and biological response of seeded human SMCs. Results indicate that elastin caused a more natural viscoelastic response via increased creep resistance and improved recoil. The outer porous layer was found to have a pore size (~90 μm) and porosity (~98%) in the ideal range to support cells. The addition of elastin was also found to stimulate mesenchymal stem cell proliferation while reducing SMCs proliferation. Spatially controlled crosslinking of the different layers was achievable due to the fabrication technique which allowed the ability to fine tune the mechanical properties of the layers to within the same range as native tissue. Ongoing research focuses on the application of *in vivo*-like cyclical strain and fluid shear stress within a custom-built vascular bioreactor to aid in the maturation of the engineered blood vessel.

S11 Role of ECM in human cardiovascular development

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In addition to cells, extracellular matrix (ECM) is one of the most important components of all tissue types in the human body. It consists of fibers and networks composed of structural proteins, such as collagen or elastin. The ECM directs cell orientation in the three-dimensional (3D) space, is essential for cell migration and affects cell communication and differentiation. Moreover, ECM proteins impact the cardiovascular cell fate and are crucial for normal tissue and organ development. In our study, we systematically analyzed tissues from human fetal, adolescent and adult hearts to monitor the temporal and spatial distribution of collagenous and elastic fibers, focusing on semilunar valves. We revealed that immature elastic fibers as well as collagenous structures are organized in early human cardiovascular development, and mature elastin-containing fibers first evolve in semilunar valves when blood pressure and heartbeat accelerate. Our findings provide a conceptual framework with the potential to lead to novel hypotheses in human cardiac valve development and disease.

S12 Scar wars

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Many years ago we demonstrated that wounds made on early reptilian, avian and mammalian embryos healed perfectly with no scar. Investigation of the cellular and molecular mechanisms underlying scar free healing and comparison with the scar forming mechanisms operational during adult wound healing identified differences which could represent targets for therapeutically improving scarring during adult healing.

A key difference was the ratio of transforming growth factor Beta isoforms present at the various wound sites. In embryonic wounds that heal without a scar, TGF Beta 3 is present at a high level (as it is a skin morphogenic factor) whereas TGF Beta 1 and TGF Beta 2 are at low levels (largely as a result of a qualitatively and quantitatively different immune response at the embryonic wound site). By comparison, adult wounds which heal with a scar have low levels of TGF Beta 3 and high levels of TGF Beta 1 and TGF Beta 2. Experimental manipulation of the ratio of TGF Beta isoforms in adult wound healing by either neutralising TGF Beta 1 or TGF Beta 2 or adding TGF Beta 3 resulted in wounds which healed with a markedly improved / absent scar. TGF Beta 3 stimulates the random migration of fibroblasts into the wound site, modulates the inflammatory response and elicits a number of other mechanisms which result in a markedly improved scar.

We transitioned these studies into early human clinical trials administering by intra-dermal injection human recombinant transforming growth factor Beta 3 into incisional and excisional wounds. Initially these were applied to matched experimental wounds under the arms of human volunteers and then into a variety of clinical settings involving treating either bilaterally symmetrical wounds, (e.g. following bilateral varicose vein removal) or long wounds, (e.g. following scar revision), where one end was treated with drug and the other with placebo (TGF Beta3 is very sticky, has very limited diffusion and is not systemically available at the doses administered).

These double blind randomised phase 2 clinical trials involved approximately 1500 subjects and showed statistically significant improvements in scarring at doses of approximately 200–500 ng per 100 μL, per linear cm of wound margin. A large phase 3 trial in scar revision surgery failed to meet its primary end point. This trial uncovered significant variation in the way in which the ends of a long wound scar healed, which is not attributable to any known factors and is assumed to be genetic. Other strategies for reducing/improving scarring based on knowledge of embryonic healing include modulation of the inflammatory profile (e.g. by IL10), decreasing the levels of TGF Beta 1 (e.g. by neutralising antibody) or preventing activation, (e.g. with mannose 6 phosphate) or modulation of other cytokines e.g. reducing the levels of connective tissue growth factor (CTGF).

Acknowledgements: MRC, BBSRC, EPSRC, Renovo.

S13 Cell curvature in the definition of leader cell identity

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Epithelial cells migrate as a cohesive sheet in a process called collective migration, which is of importance in wound healing, cancerous invasion, and development, including branching morphogenesis (a form of epithelial morphogenesis that plays a role in lung and kidney development, among other organs). Cells take on different identities depending in part on their position within the sheet. Some 'leader' cells can be seen to be visibly motile, with pronounced lamellipodia at their free edge. In contrast, following cells (beside and behind the leaders) show little to no lamellipodial activity.

The geometry of cells cultured in two-dimensions *in vivo* has been shown to affect their protrusive activity, and we have demonstrated a relationship between the degree of curvature to which cells are exposed, and lamellipodia formation. This is interesting as it argues for a simple underlying rule controlling branching morphogenesis, with small differences in curvature being amplified with a feedback mechanism. In silico modelling supports this hypothesis, as relating protrusion to curvature is sufficient to produce branching structures.

This relationship appears to not be due to the action of secreted factors, and we are currently dissecting the intracellular tension-producing and -sensing apparatus to identify the key elements responsible. This work is facilitated by the use of printed substrates which constrain cultured cells in two dimensions, and in zones of defined curvature.

Acknowledgements: We gratefully acknowledge the support of the Anatomical Society.

S14 Mesenchymal stem cell therapy: host response and mechanism

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Adult mesenchymal stem cells (MSCs) isolated from bone marrow and a variety of connective tissues have been extensively tested in the treatment of bone and cartilage repair and in osteoarthritis (OA). In addition, fully allogeneic transplantation of human MSCs is tolerated in the immunocompetent host and allogeneic therapy has been effective in the treatment of graft-versus-host disease. However, there are many aspects of the biology of MSCs that are poorly described and a more exhaustive characterization is necessary to exploit these cells fully in the context of tissue repair. Adequate translation of MSC therapy will only be successful if the following are addressed: (i) development of new cell-specific markers, (ii) deciphering the therapeutic mechanism of action and unravelling the paracrine signals that contribute to tissue repair, (iii) understanding clonal heterogeneity in cultured populations, (iv) ensuring that batch variability is controlled and (v) understanding the nature of host immunomodulation by transplanted MSCs and allogenicity. Further studies are also necessary to gain a comprehensive understanding of how MSCs may contribute to cartilage repair and how they may impede the progression of OA. This presentation will address aspects of the characterization of MSCs and will also discuss their use in cartilage repair models.

S15 Gene-activated scaffolds as effective non-viral delivery platforms for enhanced bone repair

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Treatments combining nanotechnology with gene and stem cell-based therapies on biodegradable extracellular matrices are increasingly showing potential in bone tissue engineering. Gene-activated matrices (GAMs) have already demonstrated enhanced localised gene delivery resulting in bone tissue regeneration. In this study, the ability of nano-hydroxyapatite (nHA) particles, developed in-house, to act as non-viral vectors for delivery of plasmid-DNA when combined with our collagen-nHA (coll-nHA) scaffolds specifically tailored for bone repair, yielding efficient GAMs, was determined. In addition, coll-nHA-dual gene scaffolds (dual GAMs) containing both an angiogenic gene, VEGF, and an osteogenic gene, BMP2, were assessed for bone healing in an *in vivo* rat calvarial defect model. FACS analysis performed on nHA-transfected rMSCs demonstrated a transfection efficiency of 12% and no transfection-related cytotoxic effects were observed as determined using a DNA Picogreen assay. When cells were applied to the coll-nHA scaffolds under osteogenic conditions *in vitro*, the dual GAMs exhibited significantly superior osteogenic potential when analysed using microCT, calcium quantification and histology compared to single-gene GAMs and non-transfected cell controls. When the dual GAMs were assessed *in vivo*, the nHA dual GAM significantly outperformed all other groups as early as 4 weeks post-implantation as determined using microCT, histomorphometry and immuno-histochemistry. This research has demonstrated the potential of using novel coll-nHA scaffolds as GAMs for therapeutic gene therapy while also being capable of simultaneously delivering numerous genes. Incorporation of both angiogenic and osteogenic therapeutic genes enabled the exploitation of the additive potential of dual gene delivery to successfully mimic natural bone healing. This study underlines the effect of specifically tailoring GAMs for bone regeneration applications and furthermore, this novel delivery system may be used for the regeneration of numerous other tissues in addition to bone.

Acknowledgements: European Research Council (ERC grant agreement n°. 239685) and Science Foundation Ireland, President of Ireland Young Researcher Award (04/Yl1/B531).

S16 Novel approaches for the maintenance and differentiation of stem cells

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The developmental potential of pluripotent stem cells is classically demonstrated by transplanted stem cells forming teratomas consisting of identifiable tissues. Such levels of cellular differentiation are currently not feasible using conventional methods in which cells are cultured on two-dimensional (2D) flat substrates. When grown as monolayers cells acquire flattened morphologies and cell-to-cell interactions are restricted. It is now recognised that to achieve higher levels of cellular differentiation and tissue organisation, it is necessary to modify and improve the physical growth environment and maintain cells in a three-dimensional (3D) state.

In this study we report the use of Alvetex technology to support the growth and differentiation of stem cells in 3D. Alvetex is a porous polystyrene scaffold designed to support 3D cell culture. We demonstrate how alternative stem cell types show enhanced growth and differentiation when cultured in 3D compared to conventional 2D models.

Specifically, we show the formation of human neural tissues from pluripotent stem cells in 3D culture using a novel synthetic retinoid. We then show how such neurons can be used to study the mechanisms of neurite inhibition to model the function of the glial scar that forms during injury to the spinal cord. This involves the study of inhibitory proteoglycans found within the scar and the suppression of their inhibitory effects using small molecules to rescue neuritogenesis. The co-culture of glial cells and neurons improves the physiological relevance of the model further, enabling the study of cellular interactions in a 3D model.

The majority of methods used to propagate and expand stem cells involve 2D cell culture. We have developed a novel approach using Alvetex where stem cells are maintained continually in 3D. Levels of stem cell markers are increased and their developmental potential is enhanced. Data will be presented to show that stem cells maintained in 3D are primed and are more appropriate for subsequently seeding into 3D models and transplantation studies.

Collectively these data underline the importance of the physical nature of the microenvironment and its role in regulating the shape and form of stem cells that can subsequently influence their growth and differentiation.

S18 Quantifying form in regenerative medicine

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In the Biomedical Sciences we deal with complex images from a wide variety of imaging modalities. The first stage is to choose the right tools for the job. Key questions include; what is the lowest magnification that allows recognition of feature of interest?, what do you want to see?, what spatial and temporal resolution is required ?, what is the biochemical composition of the material? Do you need to label or stain? For unstained material contrast microscopy can be used microinterferometry and Spatial light interference microscopy can allow quatitative estimation of dry mass. Dark field microscopy can be used to visualise nano particles in cells. Polarization contrast microscopy allows visualisation of collagen anisotropy. Differential contrast microscopy combined with traditional stains such as acridine orange can be used to visualise gene delivery products. Structured light, confocal, multiphoton and the super-resolution microscopies are providing vital insights into the form and function of cells. In dynamic live cell imaging is your system fast enough to capture the events. In Medical Imaging a Key Challenge is to bridge the resolution gap and to determine which microscopic method provides the most functionally relevant benchmarks for interpreting clinical images. Stereology is providing a spatial framework upon which to lay the new physiological and molecular information. Other technologies including Confocal Raman Spectroscopy and Photo acoustic microscopy are becoming accessible. These tools and techniques should allow us to quantify molecular activity and place function in a structural context.

S19 Establishment of an *in vitro* cornea 3D model – a stepping stone to environmental reprogramming of skin epidermal cells into corneal epithelium

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Corneal epithelial stem cells, also known as limbal stem cells (LSCs), can become damaged in chemical burns to the eye, creating the blinding disease of LSC deficiency. The culture of LSCs *in vitro* and their transplantation has been shown to successfully regenerate the corneal epithelium. Other techniques generating human limbal epithelium for transplantation have been explored, however the LSC pool is limited; thus other tissue sources containing autologous stem cells have been proposed for regenerating the corneal epithelium. This study focused on the establishment of an *in vitro* 3D model of the cornea, paying particular attention to the corneal stroma, which makes up ~90% of the corneal thickness. The stromal keratocytes native phenotype is crucial for a functional and transparent cornea. Therefore we aimed to re-establish the *in vivo* epithelial-mesenchymal interactions taking place between the corneal epithelium and its underlying stroma through the use of a 3D hanging drop culture model. Once established, it will enable us to understand the signals involved in corneal development and maintenance.

Methods: Epithelial cells and stromal keratocytes were isolated from rabbit corneas. The corneal epithelial cultures were initiated with mitotically inactivated 3T3 mouse fibroblasts using established protocols. Stromal cells were established from explants. Single cell suspensions of stromal keratocytes were put into hanging drop culture to establish 3D spheres, and their expression compared with 2D cultures using qRT-PCR and immunohistochemistry. The stromal spheres were subsequently coated with corneal epithelial cells and cultured for up to 14 days. Double coated corneal spheres were frozen in OCT for cryo-sectioning and immunohistochemistry.

Results/Conclusion: Results suggest that corneal stromal keratocytes restore *in vivo* phenotype signature expression when changed into the 3D model from normal monolayer cultures. They lose smooth muscle actin expression and upregulate expression of other markers representative of corneal stromal cells *in vivo*. The 3D stromal cultures also provide the core of the new 3D corneal model, in which epithelial cells attach and express typical cornea markers, indicating epithelial-mesenchymal cross-talk. We aim to develop the work to environmentally reprogramme skin epithelial cells to corneal epithelium by interacting them with corneal stromal cells in the 3D model.

S20 Tissue engineering articular cartilage and bone with form and function

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Osteoarthritis (OA) affects millions of adults worldwide and is the leading cause of chronic disability in the United States. In recent years there has been increased interest in the use of cell based therapies for the treatment of small focal cartilage defects. While significant progress has been made in this field, realising an efficacious therapeutic option for the treatment of OA remains elusive and is considered to be one of the greatest challenges in the field of orthopaedic medicine. This talk will review our attempts to use mesenchymal stem cells (MSCs) to tissue engineer articular cartilage and bone grafts with comparable form and function to the native tissue – a key step in the development of new therapeutic options for the treatment of OA. By systematically investigating how MSCs respond to their biochemical and biophysical environment, we have been able to develop tissue engineering strategies to generate cartilaginous constructs with a zonal composition mimicking aspects of the native tissue. Furthermore, we have also been able to generate complex tissues, such as the bone-cartilage interface, by designing tissue engineering strategies that recapitulate aspects of the normal long bone developmental process. The talk will conclude with a demonstration of how we might ultimately scale-up such tissue engineering strategies to potentially regenerate entire diseased joints or replace whole bones.

Acknowledgements: Funding from the European Research Council and Science Foundation Ireland.

S22 The effect of hypoxia on endothelial and chondrogenic differentiation of human amniotic fluid-derived stem cells (hAFSCs) for use in orthopaedic tissue engineering

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hAFSCs represent a promising stem cell source for tissue engineering due to their pluripotentiality, accessibility and high renewal capacity. This study seeks to engineer, *in vitro*, therapeutic constructs for bone and cartilage repair using hAFSCs in combination with collagen-GAG scaffolds from our lab. We propose that exposure of cell-seeded scaffolds to hypoxic conditions (therefore activating the Hypoxia Inducible Factor-1 pathway) might create an environment more appropriate for *de novo* vascular or cartilage tissue formation within the construct. In order to investigate the effect of hypoxia on endothelial and chondrogenic differentiation, hAFSCs were cultured in hypoxic (2% O_2) conditions on either collagen-chondroitin sulphate (coll-CS) scaffolds for endothelial differentiation, or on collagen-hyaluronic acid (coll-HyA) scaffolds for chondrogenic differentiation.

hAFSCs differentiated in hypoxia presented a endothelial gene expression profile more closely resembling that of endothelial cells than hAFSCs differentiated in normoxia. This was paralleled by enhanced VEGF protein production and increased tubule formation, indicating the beneficial role of hypoxic exposure in endothelial differentiation. Moreover, chondrogenesis of hAFSCs seeded on coll-HyA scaffolds was significantly enhanced when cultured in hypoxia, as evidenced by increased sGAG production as well as accelerated expression of early stage chondrogenic genes (e.g. Collagen II). Cartilage matrix deposition within the scaffold was also significantly enhanced.

The results of this project indicate the suitability of hAFSCs for use in a number of areas of regenerative medicine as well as illustrating potential of utilising a low-oxygen environment to enhance the development of engineered constructs.

This work was funded by the HRB in Ireland under Grant No. PHD/2007/11.

S23 MCP-1 promotes mesenchymal stem cell migration via Gbg, PI3K and ROCK dependent mechanisms

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Transplanted mesenchymal stem cells (MSCs) have the capacity to migrate to sites of injury. Despite the clear significance of migration in the therapeutic activity of MSCs the mechanisms governing migration are not well understood. We found that MSCs, delivered intravenously to rats after induction of myocardial infarction, migrated specifically to the ischemic zone. MCP-1, secreted by the injured tissue, played a central role in the directed migration of the transplanted cells. While the role of MCP-1 as an injury-specific chemoattractant for MSCs has been described, the precise mechanisms governing migration in response to MCP-1 have not been well-resolved. Firstly, we aimed to characterise the cellular mechanics mediating MSC migration. Using high-resolution confocal microscopy and a live cell system incorporating a pre-formed gradient of MCP-1, we found that directional migration was supported by a polarised phenotype and distinct changes in actin and a-tubulin dynamics. By using a specific inhibitor for the Rho kinase, ROCK we found that ROCK is essential for both tail retraction and lamellipodia formation in MSCs. To elucidate the complex array of intracellular signals that may mediate migration in response to MCP-1, we used defined inhibitors to perturb receptor activation and specific points of the downstream signalling cascades. As a result we have

deciphered the critical mediators of migration in MSCs. A detailed analysis of the MCP-1 receptor, CCR2, revealed that CCR2 and the adapter molecule, FROUNT, are dynamically regulated over time in a manner that is dependent on the bg subunits of GPCRs. In fact, bg subunit activation contributes to all of the downstream pathways that ensue and are essential for MCP-1 induced MSC migration. Specifically, we define a role for PI3K, ERK1/2 and the Rac effector, PAK. Together these results provide novel insights into the important mechanisms orchestrating MSC migration. By following a systematic approach we have been able to map a path of MSC migration to a therapeutically relevant chemokine. As a result we have identified potential targets in MSCs that could be exploited to improve methods for systemic delivery, homing and retention of MSCs in injury or disease settings.

EDUCATIONAL SYMPOSIA – THE DARK ART OF LEARNING OUTCOMES

E25 The dark art of learning outcomes

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The signing of the Bologna Agreement in 1999 was one of the most important developments ever to take place in higher education in Europe. The overall aim of this agreement is to improve the efficiency and effectiveness of higher education in Europe in terms of academic standards of degrees and quality assurance standards. One of the main features of the Bologna Process is the need to improve the traditional ways of describing qualifications and qualification structures. As a result of this, all programmes and modules in the 46 countries that have signed up to the Bologna Agreement must now be described using the common language of Learning Outcomes. Programmes in many other countries are also being written in terms of learning outcomes to facilitate mutual recognition and enhance student mobility.

This presentation will explain the concept of learning outcomes, will discuss the background developments leading to the recent increased emphasis on Learning Outcomes in education, and will outline with the aid of many examples the recommended guidelines in the literature for writing learning outcomes. The distinction between terms such as aims, objectives, learning outcomes and competences will also be discussed. Any confusion in your mind about these terms will disappear and everything will become crystal clear! There will also be a workshop where participants will gain practical experience in applying these guidelines to writing learning outcomes for the anatomy curriculum.

E26 An exploration of anatomists' and clinicians' views towards the use of body painting in medical education

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Aim: The aim of this study was to explore the educational value of Body Painting, as perceived by anatomists' and clinicians', concentrating on perceived advantages and disadvantages, issues of practicality, and the educational theories underpinning its use.

Method: A Grounded Theory Approach was used to collect qualitative data from 25 expert educators across 11 institutions (UK, Ireland, USA). Purposeful and snowball sampling was used to recruit participants until thematic saturation was reached. Data was collected using semi-structured interviews. Transcripts were analysed using open, axial and selective coding throughout iterative cycles of constant comparison of the evolving data set.

Results: Several themes emerged from the data: The modality provides a hidden curriculum, whereby students develop professionalism, confidence, cultural awareness and empathy by tacit learning. Students are taught about emotional socialisation and undergo subliminal learning. Body painting provides an opportunity for identity formation of the student teacher interaction, having powerful effects on professionalism. Time constraints are major limitation of its use. Cultural issues complicate the implementation of body painting. Perceived ideas are often held by staff, in terms of student acceptability and appropriateness of the modality, when considering its use. It is recommended that staff should take part in a Body Painting session, where the tool has previously been implemented, when considering its use. Body painting creates a fun learning environment where students learn surface anatomy without realising. The visual and kinaesthetic nature of the task promotes memory retention. Artistic ability is not a limiting factor to this method. Clear instruction sheets, facilitation and purposeful explanation of the task is essential for success. Body painting is a surface anatomy teaching modality. Body painting is an adjunct to anatomical education, contributing an excellent modality for specific anatomical teaching themes; it is best used in conjunction with other methods and cannot be used solely for teaching anatomy. Body painting can be used to bridge the gap between anatomy and clinical skills.

Conclusion: Body painting is a useful tool for teaching surface anatomy; however, its power lies within the unintentional learning outcomes associated with its use, namely the hidden curriculum.

E27 Learning outcomes: a tool for informing the design of computerized anatomy educational tools

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Learning outcomes (LO) have become relevant to educational policies and are vital to the design and development of educational content. They could be seen as what students should know at the end of their learning activities. They are therefore needed in designing computerized content for anatomy education. An initial review of literature related to the development of Computerized Anatomy Educational Tools (CAET) provided little evidence to show that these outcomes were used to inform their design.

Designers of interactive computer systems have, for two decades, relied on User Centered Design (UCD) methodology in creating effective and efficient systems. UCD primarily involves placing the user and their task at the center of the design process. This is in line with the current student-centered and outcome-based approach in education policy. Our analysis of UCD showed that Learning Outcomes could be represented using task analysis method. Task analysis involves the study of what users or learners are required to do in order to achieve a task. It enables the designer understand the information flow in an electronic tool, which is necessary in developing appropriate system features and functions. Thus LO could be used to inform the design of learning tools.

We applied task analysis in creating a CAET. Learning Outcomes were acquired mainly from two accepted curricula and were broken down into individual tasks that the users needed to accomplish. A flowchart mapping individual functions was developed and used as a guide in making design decisions. This process resulted in a novel interface and interaction design with notable benefits during and after the tool's development. The LO guided the design process and the choice of functions, particularly during the brainstorming phase. This process ensured that the information represented in the CAET was not just transcribed from a non-electronic medium (print), but was designed to suit the new electronic medium and avoid the constraints present in printed materials. We therefore propose that LO could be viewed not only as a means of testing the competencies of learners but also to determine the suitability of the design of various educational tools, especially CAET.

GENERAL SESSION
ORAL COMMUNICATIONS

G28 Integration of anatomy studies in problem- based learning

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In 2006/2007 in our university introduced problem- based learning (PBL) in medicine studies to interconnect fundamental sciences with clinical disciplines and change the traditional teacher – centred study to student study -centred approach. Before this change we had traditional study system, where students studied anatomy the first three semesters and knowledge was evaluated by oral- practical and theoretical exam. Now students are studying anatomy from I to V year and VI year students are preparing their masters degree thesis, some of them do it in Anatomy Institute. Thus time of anatomy studies became longer and it gave better possibilities to master knowledge.

In PBL medical curriculum anatomy is incorporated in two ways. The first way is traditional systemic studies during first year. There students receive basic knowledge for the next step studies in PBL. In the first year they have anatomy in two semesters with final exam. It consists of oral- practical and written- theoretical assessment with MCQ part. The final mark is average of these two assessments- (P 50% + T50%) $\times$ 0.5. Anatomy learning outcomes are uni-disciplinary, mostly oriented at acquisition and understanding of knowledge.

The second way – anatomy is integrated in preclinical (II–III) and clinical (IV–V) year modules. Anatomy here is integrated in multi- disciplinary learning outcomes, oriented at integration (synthesis) and practical application of knowledge. This way it is possible to compare results of anatomy studies by these two approaches. Main aspect of obtained results in traditional system was: quality and quantity of student's knowledge, but now other concerning aspects become important: integration of knowledge, synthesis with other disciplines and creativeness in application of acquired knowledge to solve preclinical problems. These skills are important for further clinical studies.

Consequently anatomy has more study hours in BPL and this study time is distributed, therefore students have better possibilities to gain anatomy knowledge and learn to apply it to solve real life and clinical problems.

G29 The changing role of anatomy education within the undergraduate medical curriculum: a multi-stakeholder analysis

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Anatomy has been an essential element of medical education for hundreds of years, supporting examination of a patient, the development of a diagnosis, and communication of these findings

to the patient and other medical professionals. Over time, anatomy education has evolved to cater for the ever-growing medical knowledge, rapid advancement of both learning and medical technologies, and changes in the structure of undergraduate medical curricula. Anatomy education has been revolutionized with reliance on preservation methods that create more life-like cadavers, use of models, imaging, simulation, as well as web-based resources to further consolidate and enhance the learning experience. This cross-section survey-based study aims to identify both student and educator attitudes and perceptions nowadays towards the role of anatomy education within the Irish and British undergraduate medical curriculum.

A quantitative questionnaire was created, assessing pedagogical domains and areas relevant to anatomy and its role in undergraduate medical education. This questionnaire was completed by the following groups: UCC undergraduate medical students [graduate-entry and undergraduate-entry programmes]; UCC medical graduates [who graduated from the UCC undergraduate-entry programme between the years 2007 and 2011]; anatomy education teaching staff at Irish and British university medical schools.

The results confirmed important differences across these three groups with respect to perceived efficacy of various methods of teaching anatomy; the role of computer-assisted learning/vs. traditional teaching methods; and the importance of anatomy education vis à vis professional practice. This study provides important insights into the role of basic and clinical anatomy within Irish and British undergraduate medical curricula which have changed greatly with respect to content and mode of delivery in the previous decade. Additionally, this study exposes the impacts of the changes in anatomy education within the undergraduate medical curriculum (particularly in an Irish context) and how it has affected the effectiveness of medical education both for students and educators.

G30 The developmental basis for the evolution of vertebrate three-dimensional mobility

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Chordates are characterised by contractile muscle on either side of the body that promotes movement by side-to-side undulation. Jawed vertebrates (gnathostomes) have refined this system: body muscle is segregated into distinct dorsal (epaxial) and ventral (hypaxial) components that are separately innervated by the medial and hypaxial motors column via the dorsal and ventral ramus of the spinal nerves. This allows full three-dimensional mobility, which in turn was a key factor in their evolutionary success. How the gnathostome system is established during embryogenesis and how it may have evolved in the ancestors of modern vertebrates, is not known.

Vertebrate Engrailed genes have a peculiar expression pattern as they temporarily demarcate a central domain of the developing musculature at the epaxial-hypaxial boundary. Aim of our study was to investigate whether Engrailed genes serve to control epaxial-hypaxial muscle development and innervation.

Investigating the chicken and mouse model representing sarcopterygians as well as the zebrafish model representing actinopterygians, we found that in all, the Engrailed expression domain was associated with the establishment of the epaxial-hypaxial boundary of muscle. Moreover, the outgrowing epaxial and hypaxial nerves orientated themselves with respect to this Engrailed domain. In the chicken, loss and gain of Engrailed function changed the epaxial-hypaxial somite pattern. Importantly, in chicken, mouse and zebrafish, loss and gain of Engrailed function severely disrupted axonal pathfinding and prevented correct epaxial-hypaxial muscle innervation.

Our work suggests that the deployment of Engrailed in the developing musculature facilitated the separate innervation and segregation of vertebrate epaxial-hypaxial muscle. Moreover, our work for the first time provides experimental evidence for how vertebrate three-dimensional mobility may have evolved.

G31 Determining the cause of selective vulnerability of motor units in a mouse model of spinal muscular atrophy

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Spinal muscular atrophy (SMA) is a childhood form of motor neuron disease characterized by the loss of lower motor neurons. SMA is caused by a ubiquitous reduction in functional SMN protein, due to the deletion or mutation of the SMN1 gene. One of the earliest pathological events in SMA is the degeneration of neuromuscular junctions (NMJs), but the rate of NMJ degeneration varies considerably between muscles. Why some motor neurons are particularly vulnerable to a ubiquitous reduction in SMN protein is unknown. We first sought to quantify NMJ degeneration in a severe mouse model of SMA in order to create a topographical 'map' of vulnerability. Motor neuron vulnerability was assessed across ten muscles in late-symptomatic Smn−/−;SMN2 mice, confirming the presence of both vulnerable and disease-resistant motor neurons. We then reconstructed whole motor neurons in young adult Thy.1-YFP-H mice, expressing YFP in a subset of motor neurons, in order to identify any correlation between morphological properties of motor neurons and their vulnerability in SMA. No correlation was found between vulnerability and any aspect of motor unit morphology, including; motor unit size, total arbor length and

branching pattern. Additionally, no correlation was found in developmental synapse elimination rate, terminal Schwann cell number, body axis position or muscle fibre type. We therefore hypothesise that molecular characteristics of motor neurons determine their relative vulnerability. To assess this, we modified retrograde tracer techniques to selectively label motor units innervating either vulnerable (tibialis anterior) or resistant (extensor digitorum longus) muscles in healthy mice. Laser capture micro-dissection was used to isolate the cell bodies from the spinal cord allowing subsequent microarray analysis to be performed on extracted RNA. We propose that this approach is a valid way to uncover characteristics of motor neurons that make them vulnerable in SMA.

G32 SMN-dependent defects in Schwann cells in mouse models of spinal muscular atrophy

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Spinal muscular atrophy (SMA) is the leading genetic cause of infant mortality. SMA is caused by mutations in the survival motor neuron 1 (SMN1) gene. Although traditionally viewed as a disease of the lower motor neuron, the ubiquitous expression pattern of SMN protein suggests that damage to other cell types, including neighboring non-neuronal glial cells, may contribute to disease pathogenesis. Indeed, previous studies have reported alterations in expression levels of myelin genes in microarray screens on SMA mouse spinal cord. Here, we demonstrate that intrinsic defects in myelinating Schwann cells contribute to disease pathogenesis in SMA by a number of *in vivo* and *in vitro* approaches. We show *in vivo* defects in myelination of peripheral nerve in two SMA mouse models, alongside abnormal maturation of paranodal axo-glial interactions. Using an *in vitro* model of isolated Schwann cells we demonstrate intrinsic, SMN-dependent defects in myelination and cell differentiation in SMA-derived Schwann cells, occurring in the absence of any influence from pathological motor neurons. DRG neuron co-cultures with SMA or wild-type Schwann cells revealed a significant decrease in DRG neurite densities when SMA Schwann cells were present. Finally, we demonstrate that Schwann cells contribute to the SMA peripheral nerve phenotype likely as a result of impairment in the physical relationship between Schwann cells and axons, due to reduced expression of laminin $\alpha 2$ in the extracellular matrix, rather than being caused by secretion of a neurotoxic substances from Schwann cells. We conclude that Schwann cells are directly affected by low SMN levels and that intrinsic defects in Schwann cells, contribute to myelination defects and peripheral nerve pathology in SMA.

G33 Remodelling bone remodelling: identification of excrescences in aged human bone

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We previously documented non-coupled formation of bone in trabecular excrescences, recognized firstly in pathological bone samples from patients with osteoarthopathy of alkaptonuria and subsequently in patients with osteoarthritis (OA), (Taylor, 2012). These structures, of which three distinct types have been observed, appear to arise from extracellular matrix deposition onto surfaces which displayed no evidence of prior osteoclastic action. Our current study aimed to analyse cadaveric knee samples for the presence of these novel structures. Three cadaveric knee samples (mean age = 88 years) without macroscopic evidence of OA were investigated. Distal femurs and proximal tibiae were sectioned coronally. Decalcified wax embedded sections were used for analysis by routine histology (brightfield, fluorescence and polarised). The results demonstrated all three types of previously described excrescences were found in all samples analysed. Their presence was seen across all areas of the bone architecture; subchondral plate down through the trabecular network, demonstrating poor integration with the existing trabeculae. Many of the identified structures had morphologies not typical of normal osteoclastic or osteoblastic action, although some demonstrated the presence of osteocytes within them. Pre-existing trabeculae and the centres of excrescences showed normal levels of fluorescence but the periphery and the surfaces of contact between the excrescences and prior trabeculae showed low levels of fluorescence. Polarized light examination revealed normal lamellar structure throughout existing trabeculae, contrasting with a lack of lamellar structure seen in excrescences. A small number of excrescences demonstrated poorly developed lamellar structure at the centre of the excrescences but no evidence of lamellar structure at the interface with the prior trabeculae. Many excrescences demonstrated a continuation with the adipocyte collagen network, suggesting that the adipocytes of the marrow space may in part be responsible for templating these structures. We have demonstrated that trabecular excrescences are not just present in overtly pathological bone but also in macroscopically normal aged human bone samples. These structures arise without previous osteoclastic action, supporting the theory that coupled remodelling is not the sole mechanism of bone internal restructuring. Furthermore the mechanism by which these structures are formed may present novel therapeutic opportunities for treatment of diseases of bone.

G34 A role for thin filament sarcomeric proteins in the developing heart

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A number of structural proteins, such as alpha myosin heavy chain and cardiac alpha actin, are known to play a crucial role in cardiac development and function. Mutations in genes encoding these proteins are linked to cardiomyopathies and congenital heart defects (CHDs). However, some thin filament sarcomeric genes expressed in the human embryonic heart are associated with cardiomyopathies, and have not yet been linked to CHDs. Using the embryonic chick (*Gallus gallus*) as an animal model, an expression profile was conducted to elucidate the expression of genes encoding two thin filament sarcomeric proteins in the developing heart. Expression profiling via RT-PCR revealed expression of the thin filament RNA from the earliest stages of heart development and protein expression was seen throughout the myocardium. Antisense oligonucleotide 'morpholinos' targeting these sarcomeric proteins, were applied to developing chick embryos in ovo. Phenotypic analysis was conducted on these morpholino treated embryos at a gross morphological and ultrastructural level. Morpholino treated embryos presented with a number of phenotypes including abnormal looping, abnormal atrial septal formation, and/or abnormal trabeculae formation in the ventricles. These data suggest that thin filament proteins expressed in early heart development play a vital role in cardiogenesis. The phenotypes elucidated from the morpholino treated embryos indicate that these thin filament proteins are candidates for screening individuals with CHDs.

G35 Mechanical characterisation of the prostate: initial results

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Benign Prostatic Hyperplasia (BPH) is a condition associated with the ageing man, caused by proliferation of the prostate stroma and glands resulting in enlargement of the prostate and subsequent outlet obstruction. This will affect over 70% of men over the age of 70 years and causes a wide range of symptoms. Recent evidence suggests that there may be a correlation between the elasticity of prostate tissue and patients symptoms. We aimed to confirm the presence of such a correlation.

We performed uniaxial tensile testing, using a custom designed tensile tester, on samples of benign hyperplastic prostate tissue harvested at Transurethral Resection of the Prostate (TURP) and compared the engineering stress, engineering strain and elastic moduli values obtained, with prospectively collected data relating to patient bladder obstructive outlet symptoms, namely the International Prostate Symptom Score (IPSS) and it's subscore, the IPSS Voiding score (IPSS-v).

Twelve Patients have been included in the study to date with a mean age of 72.1 years ($\pm$6.83) The mean IPSS score was 12.9 ($\pm$6.06) and the mean IPSS-v score was 7.08 ($\pm$3.77). Elastic Moduli (EM) were calculated for each patient using prostate samples with smallest width:length ratio, at 10% and 25% strain.

There was a moderate correlation between the IPSS-v and the EM at 25% strain ($r = 0.657$, $P = 0.015$) and a correlation that approached significance for IPSS-v and the EM at 10% strain ($r = 0.529$, $P = 0.63$). There was no correlation between the IPSS and the EM at either 10% strain or 25% strain ($r = 0.106$, $P = 0.73$ and $r = 0.229$, $P = 0.452$).

Our results show that there is a correlation between the mechanical properties of the prostate and the symptoms experienced by a patient. We expect our results will increase in significance with further patient recruitment. This information can be used to develop new methods of assessing the benign symptomatic prostate.

G36 The role of PCP genes in maintaining the corneal epithelium

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Planar cell polarity (PCP) is the mechanism by which cells organize themselves within the plane of a tissue, perpendicular to the apical-basolateral cell axis. PCP genes are the guidance cue for migrating cells during convergent extension and neural tube closure. Polarised cell migration is required for corneal epithelial integrity and in the present investigation the maintenance of the corneal epithelium was hypothesised to be controlled by PCP genes. The expression of multiple core PCP genes was demonstrated in the corneal epithelium for the first time by RT-PCR, Western blot and immunohistochemistry. In order to determine the role for PCP pathways in normal homeostatic corneal epithelial cell migration, *Looptail* mice, mutant for the core PCP gene, *Vangl2*, were bred to mice carrying an X-linked LacZ transgene (*XLacZ*) which can be used to trace the radial tracks of migrating cells across the cornea. Analysis of the *LacZ*$^{+}$ patterns in *Vangl2*$^{Lp/+}$ *XLacZ* mice showed roles for PCP pathways in polarising the adult corneal epithelium. Furthermore, tissue-specific deletion of *Vangl2* affected the stratification of the corneal epithelium, suggesting additional roles in controlling apical-basal polarity.

FORM AND FUNCTION IN REGENERATIVE MEDICINE POSTER COMMUNICATIONS

A3 Improving airway *in vitro* models with tissue engineering: three-dimensional culture of bronchial epithelium on collagen-based scaffolds

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In order to develop efficacious and safe therapeutics for the treatment of chronic disease, improved, physiologically-relevant three-dimensional (3D) *in vitro* models are required to improve *in vitro-in vivo* correlations. Respiratory drug delivery has the potential to treat both local and systemic disease but it currently lacks such a model. Tissue-engineering strategies offer a means to improve *in vitro* airway modelling by supporting long-term growth and differentiation of respiratory epithelia, and significantly enabling the co-culture of multiple airway cell types together. In this study, freeze-dried porous 3D collagen-glycosaminoglycan (CG) scaffolds were assessed for their ability to support the growth and differentiation of a functional airway epithelium. Calu-3 bronchial epithelial cells seeded onto scaffolds were cultured at either an airway-liquid interface (ALI) or liquid-liquid interface (LLI) for 28 days. DNA quantification revealed that Calu-3 cells proliferated on the scaffolds in both conditions, with significantly increased growth at ALI culture. Immunostaining and histological analysis detected the presence of tight junction protein and mucin, all indicative of barrier formation; notably, mucin staining was much stronger with ALI culture. Quantitative reverse transcriptase-polymerase chain reaction confirmed increased mucin production in ALI cultures compared to LLI culture and conventional Transwell monolayer culture, with increased MUC5AC expression at days 7, 14, and 21. Reduction of scaffold mean pore size improved cell adherence at the ALI. Overall, we have shown that the CG scaffolds can support the proliferation and differentiation of airway epithelial cells, particularly in ALI culture, and provide effective templates for further scaffold design and tailoring as *in vitro* airway models.

Acknowledgements: This project is funded by the Programme for Research in Third Level Institutions Cycle 5 & the European Regional Development Fund.

A4 An investigation of the ability of IL-10 overexpressing mesenchymal stem cells (MSCs) to delay or prevent osteoarthritis progression in mice

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Osteoarthritis (OA) is a disabling degenerative joint disease affecting synovial joints, which is characterised by loss or damage of articular cartilage. Synovial inflammation is believed to contribute to both symptoms and disease progression. The use of adult mesenchymal stem cells (MSCs) to modulate/prevent disease progression is an area of active investigation. In recent years there has been a paradigm shift in the mode of action of MSCs from direct tissue formation to modulation of the local environment by release of anabolic and immunomodulatory factors. Given the role of inflammation in OA it is logical that anti-inflammatory factors such as interleukin 10 (IL-10) might also delay or prevent OA progression. The aim of this study was to investigate the ability of adult MSCs overexpressing IL-10 to delay or prevent OA in a collagenase induced model. One unit of collagenase (in 6 mL) was injected into the knee joints of C57BL/6 mice twice over 2 days. One week later animals were treated with intra-articular injection of 20 000 adult human MSCs (in 6 mL saline) or MSCs overexpressing adenoviral Epstein Barr Virus IL-10. Adnull expressing MSCs, vehicle and adIL-10 alone were used as controls. After 7 weeks legs were harvested for histology and draining lymph nodes were taken to assess the effects of MSCs and IL-10 on T and B cell populations. Following treatment there was a decrease in the presence of CD4 and CD8 positive T cells in the popliteal lymph nodes in IL-10 transduced MSC treated animals compared to other groups. Scoring of the degree of arthritis is underway with regard to effects on serum cytokine production and OA progression as determined by the OARSI scoring of whole joint histological sections.

A5 Expression of CD68 positive macrophages in mesenchymal stem cell and schwann cell loaded scaffolds in a transection model of spinal cord injury

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Spinal cord injury is a debilitating disease which can have a profound impact on a patient's quality of life, and a huge financial cost for healthcare systems. The injury is characterised by a primary traumatic phase and a secondary inflammatory phase. Many of the current treatments focus on reducing the traumatic injury but success is often limited. Modulation of the inflammatory phase via cell transplantation has been proposed

as one potential therapy. This can be achieved using cell loaded polymer scaffolds. However, much remains to be elucidated about the complex interactions of the immune system before we can expect successful modulation. In this study we determined the expression of macrophages within a polymer scaffold comprising seven channels, which were loaded with matrigel, mesenchymal stem cells (MSCs) or schwann cells. Scaffolds were implanted within transected rat spinal cord and harvested after 4 weeks. We hypothesised that there would be a difference in the pattern of inflammatory cells present in MSC and schwann cell loaded OPF scaffolds in comparison to control (matrigel) scaffolds. Immunohistochemical staining for macrophages was carried out on paraffin embedded sections using CD68. Staining was carried out within the three animal groups at three levels within each scaffold (¼, ½, ¾). Fluorescence microscopy and Image J analysis were carried out. There were significant results for comparisons within the MSC group but no significant findings emerged in comparisons between different animal groups. It was also observed that there was a degree of co-localisation between MSCs and macrophages within channels. Macrophage infiltration was seen within every animal group, at varying intensity. Given the tendency towards pro-inflammatory effects of macrophages at 4 weeks post-injury, our results suggest increased inflammation associated with MSCs. Further study is ongoing to establish the relative proportions of pro- and anti-inflammatory macrophages present.

A6 The effect of substrate stiffness and delayed cell differentiation on bone tissue formation within gelatin scaffolds

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Cell-seeded tissue engineering scaffolds have a limited capacity to regenerate bone *in vivo*, arising from cell death in scaffold cores and failure to integrate with the host tissue. Cell differentiation at the scaffold periphery produces a matrix, which acts as a barrier to remodelling and osseointegration (Lyons, Biomaterials. 2010). Cellular distribution can influence the consistency of tissue formation (Keogh, J. Cell tissue res., 2010) and scaffold stiffness affects cellular distribution (Haugh, Tissue Eng. Part A, 2011). Optimal seeding and differentiation might overcome the limitations of cell seeded scaffolds. The objective of this study is to investigate whether (i) a delay in cell differentiation and (ii) an optimal scaffold stiffness, can enhance cell distribution and mineralisation of scaffolds. Gelatin glycosaminoglycan (GG) scaffolds were produced by freeze-drying and these were crosslinked with varying concentrations of EDAC to produce scaffolds of different mechanical stiffness (0.3, 0.8, 1.8 kPa). Scaffolds were seeded with 2×10^6 MC3T3 cells and then were either (i) allowed to differentiate immediately, through addition of osteogenic growth factors to cell culture media, or (ii) cell differentiation was delayed for 7 days, during which time cells proliferated in expansion media. Both groups were cultured from Day 7 in osteogenic media up to 28 days. Biochemical and histology analyses are performed to determine cell number and distribution and mineralisation at specific timepoints (0, 7, 21 and 28 days). By day 7, there was significantly higher ($P < 0.05$) cell numbers in the 0.8 and 1.8 kPa groups (1.3×106, 1.4×106), compared to the 0.3 kPa group (1.8×105) and all groups at Day 0. A significant decrease ($P < 0.05$) in cell number occurred in the 0.3 kPa groups between Day 0 and Day 7 (6×105 vs. 1.8×105). No significant difference in cell number was observed between delayed differentiation and immediate differentiation scaffolds by day 7. The results of this experiment showed that scaffold stiffness can determine cell number throughout GG scaffolds. Delayed differentiation was not shown to affect cell numbers, but ongoing studies are being conducted to quantify distribution and mineralisation at later timepoints to investigate whether these properties are enhanced by a delayed differentiation approach.

A7 Control of muscle stem cell maintenance vs. differentiation in the chicken embryo

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Few human organs can regenerate after disease or injury using specific stem cells. Following an asymmetric cell division, tissue specific stem cells give rise to a differentiating cell that rebuilds the injured tissue, and a renewed stem cell that repopulates the stem cell niche. This infers that these stem cells are committed to a specific differentiation pathway and fate, but their stem cell status is protected. How this balance is regulated on a molecular level is not known. Yet understanding the underlying mechanism will provide us with the knowhow and tools to generate and manipulate tissue specific stem cells for therapy of organs that currently are unable to regenerate.

We aim to investigate the genetic control of precursor cell and stem cell maintenance, muscle stem cells as *in vivo* models. Somites lay down the primitive musculature as well as providing the embryonic muscle stem cells. Embryonic muscle stem cells build the entire fetal and postnatal musculature and provide the adult muscle stem cells known as satellite cells. To explore the protection of their precursor/stem cell states, we misexpressed members of the MyoD family of transcription factors. These factors are commonly referred to as muscle regulatory factors (MRF) as *in vitro*, they can drive myogenic and non-myogenic cells into muscle differentiation, and in the neural tube *in vivo*, they can suppress the neurogenic and activate the myogenic program.

In the first series of experiments, we electroporated MRF constructs into myogenic precursor cells when they were first laid down during gastrulation and somitogenesis. In the second series of experiments, we electroporated the MRF constructs into the prospective embryonic muscle stem cells. Embryos were analysed for the premature expression of endogenous MRF genes and for the presence of sarcomeric myosin. We found that MRF were able to activate other MRF genes but they were not able to drive cells into terminal differentiation. We thus conclude that *in vivo*, the precursors and muscle stem cells are actively protected from premature differentiation.

A8 Investigating the potential of chitosan as a gene delivery vector in the development of a gene-activated matrix for bone regeneration

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Gene-activated matrices (GAMs) have shown potential in localized gene delivery resulting in bone tissue regeneration. Chitosan (CS) is a natural cationic polymer which shows promise as a gene delivery vector as it is biocompatible, biodegradable and capable of intercellular delivery of nucleic acids. The objective of this study was to assess the potential of polymeric (Mw 160 kDa) and oligomeric (Mw 6 kDa) CS for the delivery of osteoinductive genes to mesenchymal stem cells (MSCs) in monolayer and on a 3D collagen-based scaffold, thereby developing a GAM for bone repair. Optimal conditions for formulating CS-DNA nanoparticles including pH, CS:DNA ratio, DNA load, complexation time and temperature were determined. Positively charged particles with a diameter of $<$200 nm that can fully complex DNA were chosen for transfection experiments. Four formulations of polymeric CS and six of oligomeric CS carrying the *Gaussia* luciferase gene (pGLuc) were assessed *in vitro* in MSCs. Sustained transgene expression of 5×10^5 RLUs was seen up to day 10 post-transfection with both types of chitosan while further prolonged gene expression to day 14 was observed in the oligomeric group. This study has led to the development of vectors that facilitate highly efficient and sustained transfection of MSCs for the first time. We are now assessing these CS vectors in 3D collagen-based GAMs and have the option of tailoring the expression of different osteoinductive growth factors, such as BMP-2 and VEGF, to create an integrated natural material-based GAM suitable for repair of critical sized bone defects *in vivo*.

Acknowledgements: Science Foundation Ireland Research Frontiers Programme (Grant No. 11/RFP/ENM/3053)

A10 Testing the differentiation potential of embryonic tendon cells towards an osteogenic lineage

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Tendon mineralisation is a common problem and has been described in many models of injury, age and even disease. Manifesting itself as a progressive calcification and ossification of the tendon tissue, these incidences can be very debilitating, limiting the ability of normal tendons. Tendons are the link providing force between muscle and bone and thus are an integral and indispensable part of the musculoskeletal system. The mechanisms behind this mineralisation have yet to be elucidated. It has been suggested the tendon cells, tenocytes, could be responsible for driving this process following certain cues and may be able to differentiate into cells of other musculoskeletal tissues.

To assess the plasticity of tendon cells towards an osteogenic lineage, tendon cells were harvested from three tendons of the embryonic chicken hindlimb (the ossifying extensor and flexor tendons and the non-ossifying Achilles tendon) and treated with bone conditioned media over a 12 day period. Growth rate, cell morphology, cell immunocytochemical profile and mRNA expression were recorded and analysed.

Following treatment, the embryonic tendon cell cultures appeared to contain two different cell types: one showing a phenotype typical of tenocytes, the other a morphologically different phenotype with a cuboidal shape and a clustering phenotype reminiscent of bone cells. An immunocytochemical and mRNA expression analysis of these cells revealed an increase in expression of bone specific proteins. We suggest that tenocytes are plastic in nature and in the presence of bone conditioned media are capable of differentiating down an osteoblastic lineage. Further investigation into the nature of the bone conditioned media and the factors affecting the cells would be of interest.

GENERAL

B12 Defining adaptation in the rat musculoskeletal system in response to changing biomechanical demands

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The musculoskeletal system is capable of phenotypic change in response to varying biomechanical demands. Documenting the nature of change and deciphering which mechanical stimuli primarily drive change is key to understanding changes in pathological and ageing processes. In this study a rodent hindlimb model was used to investigate load related changes of bone morphology and associated patterns of force propagation were modelled in silico; 10 rats underwent surgery. Under strictly aseptic conditions miniature neuromuscluar stimulators were implanted in to the peritoneal cavity and connected to the common peroneal nerve. This provided stimulation to the muscles of the anterior compartment every 30 s. After a period of 28 days the animals were sacrificed. All experiments were carried out in strict accordance with the Animals (Scientific Procedures) Act of 1986.

The hindlimbs were imaged using both conventional and I_2KI contrast enhanced microCT, which allows simultaneous visualisation of the bone and muscle. This data were then used to evaluate changes in bone geometry and mineral density and also to establish the pattern of strains across the tibia by creating 3D computational models of the loading regime for Finite Elements Analysis (FEA).

The stimulated limbs showed a significantly lower bone mineral density in the anterior-distal region of the tibia compared with

their contra lateral control. In this region there was also a significantly larger cortical thickness in the experimental limbs compared with their contra lateral control.
The FEA confirmed that the region of principle morphological change correlates with a region of high stress within the bone.
These results show that an altered loading regime can result in significant changes in the morphology of the musculoskeletal system, and that this change appears to be restricted to regions most affected by the increased loading. This FE model can now be modified to replicate different disease and aging processes and therefore provide valuable insight in to biomechanical implications of a loading regime in differing phenotypes.
PV was supported by scholarship from the Institute of Ageing and Chronic Disease, University of Liverpool and the authors would like to thank the Anatomical Society for funding attendance to this conference.

B13 New insights into the three-dimensional structure of the murine epidermal basement membrane

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The epidermal basement membrane (BM) is an essential component of the dermal epidermal junction, which ensures skin stability and acts as a barrier to cells and nutrients. Despite the existence of several models, the supramolecular aggregation of the BM components *in vivo* has not been elucidated to date. Such knowledge would be essential in a better understanding of BM function.
Here we present first high resolution SEM observations in murine skin *in vivo*. Samples were obtained as a surplus tissue from control mice sacrificed at the University of Münster or at NUI Galway in the context of other projects. All animal work was conducted in line with the local ethical requirements. Frozen samples were thawed and incubated overnight in high molar salt. Afterwards the epidermis was peeled. TEM showed that it was possible to expose the BM surface, which at low magnification appeared regularly waved. At higher magnification (10–15 000×) it was possible to resolve a uniform punctuate pattern which became clear at 35–50 000×. This consisted of regularly distributed globular structures of 10–30 nm in size. Between the globules, pits smaller than 10 nm were visible. Some of the globular structures were organized in clusters sized up to 200 nm and clearly protruding from the surface. Using controlled enzymatic treatment (elastase and pepsin) it was possible to reduce the roughness of the surface and expose a layer of elongated, winding, and uniform strand-like structures of about 100 nm of thickness forming a compact network. Between these strands, slits and fissures were visible, some of them being formed by loops of the strands. The strands were also provided with globular structures. After more intensive enzymatic treatment less compact and less rough strands with higher winding and loops were evident. In some locations it was also possible to enzymatically erode the surface and reach the upper layer of the dermal banded fibrils. Some banded fibrils were seen intermingling with the strands of the BM.
This is the first description of the 3D-architecture and supramolecular assembly of a BM. Further studies are being conducted in order to gain more insight into the molecular composition of the above structures.

B17 An analysis of connexin 43 expression in term myometrium and the effect of maternal BMI and age

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Increased BMI and increased maternal age are associated with several adverse outcomes at parturition, which may be related to dysfunctional contractility. Gap junctions are specialized intercellular channels linking the cytoplasm of adjoining cells. They are involved in many cell processes including the propagation of action potentials among cells in the myometrium. In myometrium gap junctions are homomeric or heteromeric complexes of the connexin proteins Cx26, Cx40, Cx43, and Cx45, of which Cx43 is the most prominent. Progesterone suppresses Cx43 expression during pregnancy, maintaining quiescence; before labour Cx43 expression is upregulated forming a functional syncytium and resulting in the coordinated contractions of labour.
Myometrial biopsies were obtained from 16 women during elective caesarean section (obese $n = 8$ normal BMI $n = 8$, not in labour). These were immunofluorescently labelled with antibodies against Cx43 and imaged with an Optigrid structured light microscope ($n = 3$). Images were analysed with Image J freeware to assess volume fraction of myometrium occupied by Cx43 and this was compared between groups with a Students *T*-test.
We observed punctate staining throughout the myometrium, which is the expected pattern for gap junctions. The volume fraction of Cx43 staining was 0.012 ± 0.005 ($n = 16$, mean $\pm$ SD) and this was not significantly affected by BMI ($P = 0.593$) or age, ($P = 0.897$).
This suggests that the dysfunctional labour observed in obese or older mothers is not associated with a decreased functional coupling of the cells.

B19 Label-free proteomics identifies peripherally-accessible biomarkers for spinal muscular atrophy

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The neuromuscular disease spinal muscular atrophy (SMA) is caused by low levels of full length, functional survival motor neuron (SMN) protein. Recent breakthroughs in pre-clinical

research have identified several potential novel therapies for SMA, including gene therapy approaches that can significantly ameliorate disease symptoms and extend life-span in mice. As a result, there is an urgent need for robust and sensitive clinical trial platforms for evaluating the effectiveness of new treatments in human patient cohorts. In particular, there is a need for sensitive molecular biomarkers to assist with monitoring disease progression. We utilized label-free proteomics to identify individual proteins in pathologically-affected skeletal muscle from SMA mice that report directly on disease status. Quantitative fluorescent western blotting was then used to assess whether candidate protein biomarkers were present in human SMA patient muscle biopsies, and to determine whether they were similarly altered in muscle, skin and blood from a second mouse model of SMA. All animal experiments were performed under license from the UK Home Office and within institutional welfare guidelines. Human biopsy samples were obtained from EuroBioBank. We identified increased expression of both Calreticulin and GRP75/mortalin as robust indicators of disease progression in muscle from SMA mice. These protein biomarkers were consistently modified across different mouse models of SMA, across multiple different skeletal muscles, and were also measurable in skin biopsies. Furthermore, initial investigation of Calreticulin and GRP75/mortalin levels in human muscle biopsy samples suggested that they were present ad measureable SMA patients. We conclude that Calreticulin and GRP75/mortalin are peripherally accessible protein biomarkers for SMA, capable of reporting on disease progression in tissue samples of muscle and skin. Further work is now required to validate these protein biomarkers in cohorts of SMA patients.

B20 Glial cells play a defining role in developmental synapse elimination at the mouse neuromuscular junction

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Synapse elimination at the neuromuscular junction is a well-characterised but poorly understood process. The close relationship between neurons and their glial cells that exists during development suggests that glia may influence this process, but their contribution to synapse elimination has yet to be fully explored. We have studied the process of synapse elimination in genetically-modified mice lacking a key glial cell protein (Nfasc155). Time-course analysis of synapse elimination in Nfasc155−/− mice revealed significant differences in the percentage of polyinnervated endplates at P7, P11 and P15 compared to littermate control mice. At P15 when synapse elimination was complete in control mice, 28% of endplates remained polyinnervated in Nfasc155−/− mice ($N = 4$, $P = 0.0009$, 0.6% in Nfasc155+/+ vs. 28% in Nfasc155−/−). Examination of the neuronal cytoskeleton in Nfasc155−/− mice suggested that Nfasc155 modulates neurofilament (NF) dynamics during maturation of motor neurons. Quantitative analysis of NF-H, NF-M and NF-L subunits revealed a ~20% reduction in NF-L in pre-terminal axons and in sciatic nerve, with no change in levels of either NF-H or NF-M. Synapse elimination was delayed in NF-L−/− mice, suggesting that NF-L dynamics contribute, at least in part, to the delayed elimination observed in Nfasc155−/− mice. We propose that glial cells play an important role in guiding development of the PNS, including the regulation of postnatal synaptic remodeling.

B24 Lateral orbital rim; open or closed in mammals

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We observed incidentally that bear and coyote do not have a lateral orbital rim in the skull. On the other hand, certain types of deer do have one. We attempted to determine which group has the orbit closed at the back by a bony bar and which has the orbit open without the bony bar at the back. The aim of this study is to determine the relationship between the presence of the lateral orbital rim and shape of the skull in certain mammals. From the literature, 15 mammals with the orbit closed at the back by a bony bar (closed group) and 18 mammals with the orbit closed at the back without a bony bar (open group) were selected. Calculation of interorbital–zygomatic breath ratio (IZR, Interorbital breath / zygomatic breath) and Nasal–skull ratio (NSR, Length of nasals / greatest length of skull) was performed. IZR of the closed group (0.73 ± 0.27) was significantly greater than that of the open group (0.35 ± 0.63, $P = 0.000$). NSR of the closed group (0.32 ± 0.97) was significantly lower than that of the open group (0.41 ± 0.67, $P = 0.023$). IZR of herbivores (0.68 ± 0.28) were significantly greater than those of carnivores (0.32 ± 0.05, $P = 0.001$) or omnivores (0.36 ± 0.07, $P = 0.005$). However, no significant difference was observed between carnivores and omnivores ($P = 0.908$). NSR of herbivores (0.32 ± 0.10) did not differ significantly from that of omnivores (0.39 ± 0.07, $P = 0.07$). In herbivores, 88.2% had a closed orbit and only 10.5% had an open orbit. In omnivores 75.0% had a closed orbit while 25.0% had an open orbit. An open orbit was observed in all carnivores.
The open group (primarily carnivores) showed a lower interorbital–zygomatic breath ratio, and here both orbits were located in close proximity. Although this bony structural arrangement is favorable for the stereoscopic vision, the total field of view particularly lateral vision becomes limited. It is my view that the opened lateral orbital rim compensates for the limited lateral vision.

B25 Do upper and lower orbital fat have a connection?

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Orbital fat plays an important role in surgery of eyelids. Camirad insisted that with resection of lower lid fat, remaining

upper lid fat migrates to a more posterior position (the rouleaux phenomenon), resulting in an even deeper supratarsal crease and a more hollow appearance of the upper lid. However, Manson stated that excision of a herniated fat pad does not result in mobilization of the globe. This study, using cadavers, was conducted to determine whether there is a true connection between upper and lower orbital fat.

A total of 39 orbits of 20 fresh Korean adult cadavers donated and permitted for study were used. Colored gelatin was injected into upper or lower peripheral fat (preaponeurotic fat) of each orbit. One week after injection, dissection was continued and connection of upper and lower orbital fat was examined.

No migration of gelatin injected into upper or to the central fat (intermuscular cone fat) was observed. However, migration of gelatin injected into upper or lower preaponeurotic fat into the entire episcleral space through the adipose orifice (AO) was observed. The three sides of the upper AO consisted of the superior oblique muscle before the trochlea, superior oblique tendon after the trochlea, and sclera. The three sides of the lower AO consisted of the inferior oblique muscle, lateral rectus muscle, and fascia between the inferior rectus muscle and lateral rectus muscle. The upper AO was located at the medial one-third of the orbital width and superior one-fifth of the orbital height. The lower AO was located at the lateral one-third of the orbital width and inferior one-fifth of the orbital height. The shape of the upper AO was a triangle with a base of 4.3 ± 2.0 mm and a height of 2.3 ± 1.2 mm. The shape of the lower AO was a triangle with a base of 4.5 ± 1.8 mm and a height of 2.7 ± 1.1 mm.

We propose that surgical obliteration of the AO might prevent migration of preaponeurotic fats. Prevention of migration of preaponeurotic fats will aid in avoidance of baggy lower eyelid or deepening of the supratarsal fold.

B27 The arterial supply of the spinal cord and its clinical implications

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Paraplegia or paraparesis occurring as a complication of thoracic or thoracoabdominal aortic aneurysm repair is a well known phenomenon, but the vast majority of elective abdominal aortic aneurysm repairs are performed without serious neurological complications. Nevertheless, there have been many reported cases of spinal cord ischaemia following the elective repair of abdominal aortic aneurysms (AAA); giving rise to paraplegia, sphincter incontinence and, often, dissociated sensory loss. According to the classification made by Gloviczki et al. (1991), this presentation is classified as type II spinal cord ischaemia, more commonly referred to as anterior spinal artery syndrome (ASAS). It is the most common neurological complication occurring following abdominal aortic surgery with an incidence of 0.1–0.2%. Several aetiological factors, including intra-operative hypotension, embolisation and prolonged aortic cross-clamping, have been suggested to cause anterior spinal artery syndrome, but the principal cause has almost always been identified as an alteration in the blood supply to the spinal cord. A review of the literature on the anatomy of the vascular supply of the spinal cord highlights the significance of the anterior spinal artery as well as placing additional emphasis on the great radicular artery of Adamkiewicz (arteria radicularis magna) and the pelvic collateral circulation.

B28 The plantar nerves of the foot

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During a dissection of the plantar aspect of the foot a rare variation was observed in relation to the trifurcation of the posterior tibial nerve (PTN) into the medial calcaneal nerve (MCN) and the medial and lateral plantar nerves (MPN;LPN). This is a variable area of anatomy in relation to its position relative to the tarsal tunnel as well as to whether the MCN is a branch off the PTN or the LPN. In the 93% of cases the MCN arises from within the tarsal tunnel in the remaining 7% it arises proximally to it. It was observed that it was in the rarer subgroup that the dissected foot belonged to.

The tibial nerve and its branches may be compressed at multiple points along its course. This could result in possible entrapment injuries such as tarsal tunnel syndrome if compression occurs in the tarsal tunnel, or joggers foot if the MCN is involved.

B29 Expression of autism susceptibility genes in the earliest stages of human cerebral cortex development

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Autism is a highly heritable but highly heterogeneous developmental condition characterised in childhood by a lack of social abilities, restricted interests and repetitive behaviours. Mutations in the synaptic genes Neurexins (NRXNs), Neuroligins (NLGNs) and (SHANKs) have all been associated with susceptibility to the condition. Irregular connectivity has been observed in the brains of autistic individuals and evidence has been presented for the involvement of the frontal and temporal lobes in particular. Previous RNA sequencing and microarray studies using human foetal brain have suggested that these genes were expressed as early as eight post conceptual weeks (PCWs) in the embryo despite the lack of synapses at this stage of development. It is therefore proposed that these genes may be responsible for functions other than synaptogenesis such as neuronal differentiation, migration and/ or arealisation of the developing cortex.

First we studied the expression of NRXN, NLGN and SHANK genes from 8 to 12 PCWs using semi-quantitative PCR. Cerebral cortex samples from 8 to 12 PCWs obtained from the MRC-Welcome Trust Human Developmental Biology Resource http://www.hdbr.org) with appropriate consent. They were dissected and RNA extracted from anterior, medial, posterior and temporal sections. Quantitative real time PCR was used to measure relative expression of 10 genes of interest relative to three reference genes.

SHANK genes showed a similar expression pattern from 8 to 10 weeks, with SHANK2 transcripts being more highly expressed than SHANKs 1 and 3. NRXN and NLGN genes were generally more highly expressed than the SHANK genes and show a gradual increase in expression over time. NRXN1 displayed the greatest increase in expression from 8 to 12 weeks as well as significantly higher frontal cortex expression at 12 weeks compared to other cortical regions. NRXN1, NLGN1 and NLGN4X showed particularly high levels of expression compared to other genes.

We have confirmed the expression of these synaptic genes at this early developmental stage. The next step is to study their expression patterns and protein localisation which will provide a more in-depth knowledge of their function during early development as well as clues into the mechanisms of brain development and its links with autism.

B30 Investigating the detailed structure of the venous drainage of the Wistar rat (*Rattusnorvegicus*), Marmoset (*Callithrix jacchus*) and human brains: a MicroCT, MRI and ESEM study using resin casting techniques

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The causal mechanism for intracranial bleeding resulting from traumatic head injuries is yet to be established, despite the implications this information could have, particularly in cases of possible child abuse. This project aims to substantiate the existence of fine subdural veins hypothesised to be the source of intracranial brain surface bleeding seen in cases of accidental and non-accidental traumatic head injuries, and illustrate their anatomical structure.

A unique modelling technique was developed to produce detailed resin casts of the cerebral microvasculature. Wistar Rats (*Rattus norvegicus*) and marmosets (*Callithrix jacchus*) were killed by overdose of pentobarbitone sodium in accordance with Schedule I approved criteria as part of the UK and international regulations of animal welfare. The blood was immediately washed out via transcardiac perfusion with pre-wash buffer, followed by 4% paraformaldehyde. Finally polyurethane (PU4ii) resin was perfused into the vasculature, and allowed to cure for 48 h. The tissue surrounding these vascular resin casts was then either macerated in 10% KOH to reveal the whole cast, or dissected to illustrate vessels *in situ*. Cadaveric material was also carefully dissected.

A combination of the following techniques was used to study the intracranial microvasculature: fluorescence microscopy, micro-computed tomography (microCT), environmental scanning electron microscopy (ESEM) and magnetic resonance imaging (MRI). Appropriate ethical approval was obtained for MRI scanning of both living human subjects and cadaveric material. The existence of subdural vessels has been shown via gross dissection of marmoset and cadaveric material. Fluorescence imaging of resin-filled rat brain histological sections also show fine vessels within the subdural space. Additionally, fine vascular networks have been illustrated through microCT and ESEM imaging of vascular casts of rat and marmoset material. MRI images of the human head *in vivo* have also shown small calibre vessels that could explain the often small volume subdural bleeding that can be seen in MRI scans in cases of non-accidental head injuries.

Results show the likely existence of subdural vessels, present across different species. Further work will allow this to be confirmed and to elucidate the exact morphological structure of these vessels, to determine the forces required to cause their rupture.

I would like to thank Lisa White and Nicola Weston for their invaluable help during this project. I would also like to acknowledge the School of Biomedical Sciences, and Faulty of Medicine and Health Sciences for my PhD Studentship at the University of Nottingham.

B31 Morphologic effects of methamphetamine administration at different dosages on the rat adrenal gland

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Methamphetamine (MA) is a synthetically derived psychostimulant drug with strong euphoric effects. As a consequence, the scientific focus has been primarily placed in the central nervous system to the detriment of other systems. The adrenal gland is involved in physiological responses for the survival of organisms after injury or infection. Determining both physiological and morphological changes in the adrenal gland due to MA exposure in an animal model can predict the effects of this drug in humans. The aim of this study was to evaluate and stereologically quantify the effects of acute, intermittent and chronic MA administration on the histological structure of the adrenal gland. Thirty-two 8-week old male Sprague Dawley rats were used. All treated animals received 20 mg kg^{-1} methamphetamine-hydrochloride by oral gavage in different patterns, however, controls received distilled water daily. The acute group received distilled water daily for 13 days and MA on day 14, the intermittent group received 5 days of distilled water followed by 2 days of MA twice and the chronic group received methamphetamine hydrochloride daily for 14 days. On day 15 all animals were scarificed by guillotine decapitation. Both adrenal glands were explanted, weighed, fixed and processed for routine histological staining. The samples were both qualitatively assessed and then quantitatively analyzed using computerized (Image Pro, Media Cybernetics) and manual stereological methods. MA exposure in all treatment groups increased the volume fraction of lipid droplet deposition, granular eosinophilic cells and necrosis in the zona fasciculata. These effects followed a gradual increase in their severity which correlate with the increase of MA exposure, being less in acute and more prominent in the chronic. MA exposure resulted in a decrease in thickness of the zona fasciculata and zona glomerulosa in all MA treated groups and of the zona reticularis just in the chronic group. Statistical significance were found for lipid accumulation, granular eosinophilic cytoplasm

and cortical thickness. These results suggest that the toxic effects of MA in the rat adrenal gland are focused in the cortex, which serves as a model for similar effects in humans.

B32 Novel hydrogels for site specific delivery of human mesenchymal stem cells and microRNA mimetics as a multimodal therapeutic for the treatment of myocardial infarction

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The European Society of Cardiology estimates that every sixth man and every seventh woman in Europe will die as a result of a myocardial infarction (MI). Current therapies are ineffective in restoring full cardiac function post-MI. Mesenchymal stem cells (MSCs) have gained attention as a restorative treatment option due to their ability to promote myocardial regeneration but their utility is limited by low levels of cell engraftment following delivery. This project aims to incorporate MSCs into a thermoresponsive hydrogel matrix in combination with pro-survival/angiogenic microRNAs (miRNA) to increase the levels of MSC engraftment at the site of infarction. MSCs were seeded within thermoresponsive chitosan/β-Glycerophosphate gels. Cells were Live/Dead stained at multiple time points to assess viability within the thermoresponsive chitosan gel. Assessment of dsDNA levels were also carried out to validate the ability of the cells to proliferate within the chitosan gel. Fluorescently tagged miRNAs were loaded into a thermoresponsive pegylated tyrosine gel and miRNA release into PBS was quantified by fluorescence spectroscopy over multiple timepoints. Results indicate that the chitosan gel is suitable for MSC delivery as the cells were viable and proliferated over the 5 day period. miRNA release within the pegylated tyrosine gel showed a sustained continuous release profile over a five day period which is favoured over simple bolus delivery. The next step is incorporation of these dual modalities within a bioactivity model to assess angiogenic potential.

B33 Anatomy and tensile strength of the abdominal head of the pectoralis major muscle in relation to transaxillary breast augmentation

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Background: The aim of this study is to elucidate the anatomy of the abdominal head of the Pectoralis major (AHPM) in relation to transaxillary breast augmentation (TBA).
Methods: In 20 hemithoraxes of fresh Korean cadavers, the width, thickness and location of the origin of the AHPM were measured in relation to the seventh rib-costal cartilage junction. Force gauge was used for measurement of the force needed for detachment of the AHPM from its origin. In each of other four breasts, an implant pocket was made first, followed by observation of the AHPM. In 92 patients operated, the AHPM was observed at its origin during performance of endoscopic TBA.
Results: Among 24 hemithoraxes dissected, the AHPM was observed in 23 (96%) hemithoraxes. Among 184 breasts operated, the AHPM was observed in 170 breasts (92.4%). AHPM originated from the rectus fascia at the sixth (60%) and seventh (35%) costochondral junctions. The width of the AHPM was 23.5 $\pm$ 5.2 mm, 15.2 $\pm$ 3.9 mm, and 7.3 $\pm$ 4.3 mm, at its origin, mid-belly, and insertion, respectively. The thickness of the AHPM at its origin was 1.6 $\pm$ 0.5 mm. The force needed for detachment of the AHPM from its origin was 23.5 $\pm$ 12.0 N. In two cadavers of mock surgery, the AHPM could limit the boundary of the implant pocket after division of costal origins. After division of the AHPM, the free inferior space was obtained.
Conclusion: In submuscular or dual plane breast augmentation, the AHPM should be cut to place the implant in the correct desired position.
Level of Evidence: Level III, Case control analysis.

EDUCATION

C34 Anatomy laboratory practical development

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In student feedback key problematic issues were raised consistently by students in their anatomy practicals in the laboratory:

1. Over-crowding in laboratory space and lack of access to specimens
2. Lack of feedback during laboratory sessions
3. No answers directly available to some questions in their laboratory workbook
4. Little access to staff to provide guidance during laboratory sessions

Greater numbers of students undertaking the BM5 Bachelor of Medicine course at the University of Southampton have also increased demand on the resources in the anatomy laboratory. A new practical was designed to resolve these issues, to integrate other subjects, in particular histology, and to use staff teaching time more efficiently.
The Nervous and Locomotor 1 (NLM1) course, the second anatomy course for BM5 Bachelor of Medicine Year 1 students, was re-designed. Major changes include increased time in laboratory sessions, decreased learning stations, consistent provision of two activities per station, decreasing the number of students at each station, increasing the number of overall teaching staff with staff dedicated to each station, and incorporating histology and feedback sessions.
Year 1 students (n = 143) were asked to complete a questionnaire to explore their experiences of the old style anatomy practicals on completion of their first anatomy course in year 1. The same

questionnaire was completed again on conclusion of the new design of anatomy practical.
Using Spearman's Rank order correlation, survey results demonstrate a very strong positive correlation (>0.99) to each of the questions assessed: sufficient access to the specimens; ability to complete all the class exercises and tasks; sufficient access to staff; sufficient space to work in the anatomy laboratory; understanding what is expected in the practical classes; obtaining feedback and checking answers.
These changes have been based on current trends in education to promote students to take a deep approach to learning. These results of the survey demonstrate that greater structure within the anatomy course, in particular the practical sessions, has improved the learning experience for the students.

C35 Blending the virtual and physical teaching environment: the potential role of the anatomy e-booklet

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Contemporary approaches to anatomy teaching combine the more traditional laboratory based methods with self-directed and computer based learning. The rapid development of mobile devices and technology has unlocked new opportunities to expand on the concept of blended learning which can offer a more personalised, flexible, educational experience. At Southampton we recently introduced a first generation e-booklet to support our laboratory based teaching experience. It contained a variety of interactive content such as; self-assessment diagrams, narrated lectures, quizzes and a discussion board, all prepared in a mobile friendly format for use inside or outside the laboratory. A longitudinal study carried out over one semester (5 months) involved 220 first year BM5 medical students studying the respiratory, cardiovascular and renal systems. Analysis of e-booklet activity from the resource platform allowed us to track student usage. Our results show that 88% of students voluntarily opted to use the e-booklet alongside the printed version. At the beginning of the semester there was an initial peak if 1100 daily visits, this then steadied to 120 per day through the semester and including vacation time. Not unexpectedly there was a peak during revision time with 360 daily visits. In exploring the students learning process a trend was identified showing that content was accessed during both scheduled and self-directed time. Interestingly, the frequency of visits to specific pages of the resource differed. Students spent 30% of time spent using the e-booklet to view interactive diagrams, 26% of time on exercises and 22% on introductory material. The average time spent using the electronic handbook was 6.4 h per sudent. In conclusion, it is clear that an e-booklet can successfully blend the laboratory and self-directed learning experience. The on-line, interactive course handbook supports autonomous and personalised learning in different environments and simultaneously facilitates more flexible learning. Further exploration into the impact of combining the virtual and physical learning environment is welcomed.

C36 Student ownership and use of electronic media in anatomy and histology learning at the University of Southampton

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Student ownership of electronic hardware, such as smartphones, iPads and desktop PCs, has increased dramatically in recent years. Generic software resources are also increasing rapidly, both within education and non-education. In this study we sought to determine: what benefits students identified for e-learning and other learning formats (lectures, practicals, tutorials etc.); what electronic hardware students owned; what software (apps, Facebook, websites) students knew of; what software students used socially; which software students used to aid anatomy learning; what software is most useful in their anatomy learning.
Medical students from Year 1 and 2 BM5 Bachelor of Medicine course were asked to complete a short questionnaire. Responses were compared between first and second year students (162 and 95 students respectively).
No statistically significant difference was realised between the 2 year groups in ranking the benefits students identified for e-learning, or for other learning formats. When comparing the benefits for e-learning against all other formats the ranking was the same in Year 1 and 2.
No statistically significant difference was identified between the two year groups' ownership of electronic hardware, their awareness and use of non-educational software. Both Year 1 and Year 2 used different software for anatomy learning and generic purposes, and this difference is statistically significant. Whilst the software students used to learn anatomy is statistically different in Year 1 and Year 2, closer examination of the data shows the top three are in the same order. The most used software being the bespoke Faculty of Medicine online software.
It was interesting discover that the students ranked the benefits for e-learning the same as other formats. Students in both first and second year seemed to use the same software for anatomy learning, with the top three choices being the Faculty of Medicine bespoke software, Youtube and interactive internet sites, in the same order. Of particular note is both that the bespoke software from the Faculty of Medicine is ranked highest, and that Youtube ranks a close second, higher than interactive internet sites. The next two choices were both textbook based resources.

C37 Pathological pots: a valuable physical and virtual resource

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Internet based audio recordings were an innovation in the second year medical student respiratory morbid anatomy teaching. Short auditory stimuli were linked with a case study

questions and photographs of anatomical specimens. These link pathological lesions directly to the signs and symptoms of disease. These were extremely popular with learners and accessible outside the laboratory environment. They were made to ensure all learners had equable access to the resources and an awareness of learner and pathologist teaching time being pressurised. They link pathological lesions directly to the signs and symptoms of disease. Evolution of this teaching compared favourably with previous 2 years evaluation of resource heavy teaching with physical interaction in the laboratory with the three dimensional specimen. Pod casts are a way of giving distilled and thoughtful stimuli in a resource conscious and accessible way. However, interaction with the physical three dimensional specimen is still invaluable and must be encouraged. This evaluation has encouraged the development of similar resources for different body systems.

C38 Pathology pots; linking educational value

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A large, accumulated collection of pathology specimens without descriptions and with minimal labelling is being developed as a shared, multi-centre teaching resource. The aims are to create a system simple to implement over a long period of time, simple to transfer between institutions, and simple for learners to work with. We are linking the physical pathology specimen with Internet-based information by tagging the pot with machine readable code.

Pathologists review pathological specimens and make a short audio recording for each, describing the visible pathology, their causes, and often including a fictitious case that aids linking of symptoms to pathology for the learner. The audio recording and relevant links are added to a custom website's database, generating a new dynamic web-page and a unique QR code that is printed and applied to the specimen's case.

Students can use a mobile device with a QR code scanning application to scan the code and be directed to a mobile-optimised website that holds the title for the pot, a small image and a 3–5 min audio description of the visible pathology.

In this way the emphasis is applied to the pathology specimen itself, aiming to encourage students to engage with the physical tissue and not solely the internet-based information.

C39 The value of clinically orientated anatomy teaching in the undergraduate medical education

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The study of Anatomy has been vital in undergraduate medical education of future clinicians. Anatomical knowledge is essential in daily clinical practice, as it is applied for instance when performing a physical examination or clinical procedure on a patient, interpreting diagnostic imaging or explaining clinical findings (Turney, Ann Royal Coll Surg, 89, 2007). In this study, we aim to evaluate the impact of attending non-compulsory clinically orientated Anatomy tutorials on the performance of undergraduate medical students. This was a cohort study performed during the undergraduate academic period between February 2012 and May 2013. The total number of first year medical students was 503 (250–253) and included those enrolled in two different academic years of the Bachelor of Medicine and Bachelor of Surgery course at the University of Nottingham. Students were offered an average of 10 supplementary weekly Anatomy tutorials per term which were all conducted in a lecture theatre, apart from two sessions which took place in the anatomy dissecting room. Their attendance was rigorously monitored and related to their academic performance measured using the overall grades individually achieved by the students in their final exams. In addition, anonymous feedback forms were collected at the end of each term prior to the exams. The attendance varied from a minimum of six to a maximum of 74 students per session. Students who attended the anatomy tutorials performed better in their final exams and appreciated the clinical relevance and aspects of the subject. In particular, among the students who obtained a below average overall grade ($<$65%), those that attended the non-compulsory sessions performed about 6% higher than students who did not attend. On the other hand, among the remaining cohort of students who achieved a grade $\geq$65%, the increase in the overall performance was about 1–3%. This study highlights that medical students benefit from a clinically orientated approach to Anatomy and find it helpful in understanding the correlation between their basic science knowledge and the clinical practice. Furthermore, it shows that by attending non-compulsory supplementary sessions they tend to have a better academic performance.