

## THE E-TERM RESEARCH LANDSCAPE

This document outlines below the strategic landscape from which the projects to be undertaken within the Landscape Award will be distilled. We have defined the landscape in terms of: 'push' - the basic science and technology challenges that provide the cornerstones upon which the regenerative medicine industry can be underpinned; two experimental platforms that provide the state-of-the-art facilities for research; and a 'pull' landscape where we outline three 'product vision' areas. Landscape Fellows will undertake two pieces of research: a directed element that contributes to the development and enhancement of the two platforms (see below) and an individual element where Fellows (in consultation with the Management Board) will draw up a self-directed translational research project from within the wider landscape.

### **PUSH - BASIC SCIENCE AND TECHNOLOGY CHALLENGES**

In order to allow regenerative medicine to reach its full potential, fundamental science and technology challenges need to be addressed by the clinical, biological and engineering and physical sciences communities. Regenerative medicine interventions arise from a range of subject areas from materials science and medical device technologies to cell biology and tissue engineering and including cell biology-device hybrids particularly to allow organ repair or replacement. Consequently, interface challenges include:

#### ***Right patient, right time, right place – right solution***

With the advent of genomics, and an understanding of disease and the effectiveness of therapeutic advances, it is becoming clearer that stratified (or even personalized), disease specific interventions will be required (see Bagnaninchi et al 2010). These will need to take account of individual patient phenotype and stage in disease pathology. Interventions will increasingly focus on early stage interventions aimed at reducing the ultimate impact of the disease and for increased cost-effectiveness. For regenerative medicine interventions this particularly requires the definition of the right scale of intervention, appropriately stratifying and replicating the host environment, definition of an optimal intervention-host interface and sharing of structure and function between the host and intervention. Inevitably this will require the generation of tissue specific structures and scaffolds, and for stem cell solutions the careful quality assurance of cell populations (Saleh et al 2010) including the sorting of minimally manipulated patient specific stem cell populations.

#### ***Translational EPS technologies enabling interventions at useful human scales***

Much of the science presently carried out in the domain is at the scale of bench biology and the culture dish with experimental replicates primarily aimed at the generation of statistically meaningful, publishable data. Little is yet at the physical size required to generate technologies and interventions at the scale appropriate for use in humans or at the product volumes required to reach a meaningful number of patients (see however Thomas et al 2009). This demands the generation of both larger physical scale and larger product volume cell and tissue processing and manufacturing technology including ultimately - in the longer term - life size organotypic culture within functional physiological bioreactors. Such manufacturing technology will have to be complemented with new metrology (and characterisation) and process sensor/biosensor and control solutions acceptable to the regulator. A key manufacturing challenge is understanding how to design processes that have extreme reliability in the presence of biological variation, in particular the definition and control of the input to the process.

#### ***Replicating biological structure and organisation, and physical conditioning at multiple and organ scales***

Realising useful tissue engineered structures demands the generation of three dimensional organisation at multiple scales from micro- to macro-structure. From an engineering and materials science perspective such structures are multi-component, self-organising composites that frequently include a number of hard-soft interfaces. Structure and tissue evolution is frequently driven by chemical, biochemical and physical signalling in tissue and cell specific niches. Mechanotransduction and other physical influences significantly affect the generation of extra-cellular matrix and its components, the differentiation of stem cells as well as angio- and neuro-genesis. Understanding how to manage nutrients and waste products in growing tissues beyond the perfusion limit is challenging as is understanding mass transfer at the organ and body length scale. Mechanotransduction must be understood through complex structures at multiple scales. This demands the generation of new engineering science (see for instance Araujo et al 2010 and Baas et al 2010) beyond conventional mechanical and chemical/biochemical engineering approaches.

#### ***Using evaluation and modelling to speed and de-risk translation***

The ambition of the product vision for regenerative medicine demands rigorous testing and evaluation. Such evaluation is likely to be tissue and disease specific, and time and resource consuming. Accelerating the translation process will require the generation of *in vitro/ex-vivo* demonstrators including physiological and anatomically full scale simulation systems. Such systems are likely to combine experimental, failure modes and effects analysis and *in silico* techniques and will draw upon emerging techniques in multi-scale multi-

functional multi-physics physical-life science complex systems modelling and analysis. Importantly such evaluations will reduce the need for preclinical animal testing. A key goal is to generate and validate technology demonstrators of evaluation platforms in which physical systems and *in silico* approaches can be reused. Each of the partners in this proposal have significant modelling capability (see for instance Green et al 2009 and Nikolaev et al 2010).

### ***Pushing the envelope - controlling the regenerative pathway in vivo***

Some regenerative medicine product strategies emphasise the use of medical device implant approaches that use bioactive or other materials to engage the body's own stem cells to assist in a repair process. It is recognised that tissue engineered and other transplants are significantly remodeled by the body. This, together with the recognition of differences between individuals and their disease stage and pathology, suggests that there are opportunities to monitor individual patient responses and use these to drive active conditioning of an implant. This clearly requires the generation of appropriate sensors and instrumentation as well as feedback and actuation techniques (see Aherne et al 2008 and 2010) including the potential for controlled drug delivery – and an understanding of the product complexity that a regulator is likely to accept.

## **PLATFORMS – BRIDGING FROM THE PUSH TO THE PRODUCT VISION**

Both DTCs have a distinctive track record of working via significant technology demonstrators. These provide a model for our way of working - the generation of technology platforms that will allow multiple individual projects and provide a mechanism for integration within our strategic themes, between the two DTCs, and between the rapidly expanding cohorts of DTC PhDs and the Landscape Fellows. We will create two new platforms within the Landscape Award, the first within the Loughborough led DTC, the second within the Leeds led DTC; these will provide the state-of-the-art experimental infrastructure within which we can maintain our research at the leading edge of international research in tissue engineering and regenerative medicine.

### ***Bioprocessing and biomanufacturing platform and technology demonstrators***

The complex task of translating the latest bioscience discoveries into large quantities of safe economic therapies is one undertaken by engineers in collaboration with clinical, biological and regulatory colleagues. For successful regenerative medicine and tissue engineering applications, human stem cells and functional tissues are required in sufficient numbers to create reproducible and cost-effective products. This is far from trivial and requires engineers both skilled in bioprocessing and able to work on systems level biomanufacturing issues. Engineering challenges include: production and processing system design, mass and heat transfer in and out of suitable artificial 3D structures including in the presence of mechanical and other signaling; expansion, differentiation and recovery of therapeutic cells at larger scales; formulation and delivery of final products and the identification of characterisation and product release criteria for cGMP manufacture. Loughborough, Leeds and Keele are particularly well positioned to address these issues since they deliver world class research in the translational space between scientific discovery and the industrial production and delivery of cell based/biological products. They have a unique experimental platform including systems for cell bioprocessing, automated cell culture, the culture of three dimensional structures under mechanical stimulation and characterization (see for example Thomas et al 2009 and Want et al 2009). Key components of this experimental platform will be evolved during the Landscape Award to enhance and extend capabilities in three dimensional tissue and organ processing; sensing, characterisation and process control instrumentation and the application of mechanical and other signaling to cell and tissue processing.

### ***In vitro simulation platform and technology demonstrators***

Like many engineering systems, functional tissue engineered products and regenerative therapies require full scale *in vitro* simulation platforms for scientific investigation, proof of technical concepts at a physiological level and to support evaluation during product development and to simulate a range of field (host) environments. Such systems also allow study of functional interactions of the intervention with the host environment and to also assess surgical delivery and monitoring technologies. Leeds (see for example Goreham-Voss et al 2010 and Vicars et al 2010) have already demonstrated the value of such simulation systems in total artificial joints and have established the largest joint simulation laboratory in the world which can generate new generic knowledge as well as product specific knowledge. EPSRC recognition of this through the cartilage tribology programme grant means full natural joint simulation systems for hip, knee and spine are being developed. Previous EPSRC funding has developed functional full scale physiological organ culture for up to 28 days of natural soft tissue constructs for ligament, bladder, and heart valves. This work has demonstrated importance of full 3D tissue structure, strain field and mechanotransduction required for effective cell differentiation and has demonstrated significance of the diffusion pathways. These platform simulation systems will be made available for full scale scientific investigations of novel regenerative therapies and interventions. Particularly important in this proposal is the Leeds/ Loughborough collaboration, which will combine large scale cell production approaches, to functional regenerative dCELL biological scaffolds (developed at Leeds), with long term organ culture in functional physiological simulation systems.

## THE PULL LANDSCAPE – PRODUCT VISION CHALLENGES

Clinical need and impact define stretch product visions to act as targets to drive technology development and demonstration. Each has combined hard and soft tissue components and demands a systematic approach.

### **Head and neck, cranio-facial defects including cleft palate**

The craniofacial region in general and cleft palate repair in particular include some of the most challenging unmet clinical needs. Patients may suffer extensive tissue loss due to cancer, trauma or common congenital birth defects such as cleft palate malformations. One of the challenges in making repairs to the head and neck region (including tissues of the skull, the face, and hard and soft palate) are that traditional prostheses are non-biodegradable - they provide immediate support and structure which can be life saving, and definitely life enhancing, but there is no possibility of these being fully assimilated into the patient. Bone, while providing an immediate repair for the hard palate unfortunately does not then grow at the rate of the growing child and can lead to distortion of the face and the jaw which require further reconstructive surgery. It is a particular challenge for children where, for example in extreme defects of the palate, there is a need for tissue for repair which is capable for growing with the rapidly developing child. We seek to reduce the need for repeated corrective surgeries as the child grows.

Sheffield have clinical expertise in tissue engineering of soft tissues, skin and oral mucosa, (see for example Moustafa et al 2007 and Bhargava et al 2008) and in developing electrospun biodegradable polymers which can be spun onto complex 3D shapes successfully. Combination of this with rapid prototyping facilities would enable bespoke implants to be made for complex surgeries. Large animal trials of craniofacial approaches are current at Keele. We propose a synergy of advanced engineering of prosthetic devices (Chandra et al 2005) and tissue engineering to deliver tissue engineered replacement tissues for the skull, nose and hard and soft palate.

While this focus is on craniofacial reconstruction on a patient specific basis, the challenges are very broad and apply to a range of tissue engineering applications spanning bone, cartilage and soft tissue. In particular we will address the challenges of integration of soft and hard tissues, matching the mechanical properties of the patients' tissues and promoting rapid vascularisation.

### **Repair of internal soft tissues for bladder, urinary system and pelvic floor to treat urinary stress incontinence**

Age and pregnancy related weakness in the pelvic floor tissues supporting the bladder leads to stress urinary incontinence (SUI) in up to 30% of women by the age of 65. The patient's tissues become stretched and weakened and this leads to prolapse of the uterus and/or vagina and urinary incontinence, particularly under any conditions of mild stress. The estimated lifetime risk of surgery for women because of these problems is 11%, thus this condition is extremely common and it also deteriorates with age. Current approaches support the weakened tissue with cell-free synthetic material whose initial performance is good but which often fails after several years of implantation. To achieve sustained repair it is clear that there must be cells present. The concept of tissue engineering for repair of prolapse and SUI is at an early stage despite the major clinical need. Sheffield University has started to develop a restorative intervention for these weakened tissues using the patient's own buccal mucosa (Bhargava et al 2008) – culturing cells from the buccal mucosa or using freshly excised buccal mucosa excised at the time of the operation in a one stage procedure. Cultured cells or freshly excised tissue will be combined with a biodegradable scaffold designed to promote tissue regeneration (de Bank et al 2007). The latter approach using freshly excised tissue will allow patients to be treated in one session in theatre, facilitating translation through to clinical practice.

However, there are considerable challenges which require a better understanding of how soft collagenous tissues respond to mechanical loading which will benefit from other expertise in our larger group. The long-term failure of female pelvic tissues to load which occurs with age and is exacerbated by pregnancy is well-accepted but actually poorly understood. In producing tissue engineered replacement materials it will be

#### **Projects in this area will**

- develop scaffolds for integrating bone tissue engineering with soft tissue for hard palate repair
- explore autologous bone marrow mesenchymal stem cells as a source of bone forming cell for hard palate repair
- fabricate hybrid implants that are capable of delivering the immediate structural strength required (which may vary from that required for replacement of a large area of the skull through to that required to replace part of the soft palate)
- develop a flexible disposable bioreactor technology capable of keeping cells alive within the constructs for up to 6 weeks
- develop advanced imaging of the 3D constructs grown within bioreactors using multiphoton confocal and optical coherence tomography

#### **Projects in this area will**

- Develop bioreactors for generating and measuring mechanical force in soft collagenous tissues as they develop over several weeks
- Develop tissues capable of withstanding regular distension
- Advance this work into small scale clinical evaluation.

important to look at the response of these tissues to loading during and post their production to get a better understanding of how they may perform post surgery.

Many of the questions we can pose - such as will production of tissues under load lead to more collagen and elastin production or to different cross-linking between collagen fibres - could be answered if appropriate bioreactors existed. This gap in our tissue engineering tool kit is arguably holding back important translational work in tissue engineering. Developing bioreactors for generating and measuring mechanical force in tissues as they develop over several weeks will be a challenge that will benefit this and other tissue engineering projects; it will lead to a knowledge of collagen production and maturation and how they relate to the biomechanical properties of tissues as they develop.

### **Musculoskeletal system - the ankle joint**

Regenerative therapies have extensive potential application in the musculoskeletal system. To date there has been clinical impact in areas such as bone grafting and chondrocyte transplantation, and approaches to minimally manipulated autologous mesenchymal stem cells are in clinical trial. Major challenges include the need for early diagnosis and stratification of patient groups, the need to consider biological variability associated with the host environment, the need to generate clinical solutions that restore whole joint function in a biomechanical and biological sense, and the need to consider an integrated systems approach to the development of regenerative interventions. Like other groups, we are working on investigating interventions in hip, knee and spine, but distinctively the Leeds team are taking a whole systems bioengineering approach to the research challenge. This includes developing real *in vitro* full experimental biological and biomechanical simulation systems and *in silico* virtual systems in which research and development of regenerative technologies can be investigated in real (technology demonstration) space. Leeds have developed, translated to clinic and commercialised novel regenerative scaffolds in the cardiovascular tissues, (see for instance Wang et al 2008) and are undertaking basic research on musculoskeletal tissue scaffolds (Stapleton et al 2008 and Ingram et al 2007). This proposal brings together distinctive capability from Keele in clinical cell therapies for cartilage in the ankle and Loughborough on bioprocessing of cells on a large scale with Leeds scaffold technology and simulation capability. In this programme we will focus on regenerative repair in the ankle joint, which does not currently have acceptable alternative joint replacements and lends itself to early diagnosis and intervention.

#### **Projects in this area will**

- investigate soft tissue, hard tissue and composite soft and hard tissue biological scaffolds
- explore advanced imaging to stratify patient groups and define the host environment during degeneration
- develop and validate functional laboratory and *in silico* simulation systems
- develop full scale functional organ culture systems in the ankle joint
- investigate combination cell matrix interventions on real scale

- Ahearne M et al, 2010, Online Monitoring of the Mechanical Behaviour of Collagen Hydrogels: Influence of Corneal Fibroblasts on Elastic Modulus. *Tissue Eng Part C Methods*, 16, 319-327
- Ahearne M et al, 2008, Online monitoring of collagen fibre alignment in tissue-engineered tendon by PSOCT. *J Tissue Eng Regen Med*, 2, 521-524
- Araujo JV et al, 2010, Dynamic culture of osteogenic cells in biomimetically coated poly(caprolactone) nanofibre mesh constructs. *Tissue Eng Part A*, 16, 557-556
- Baas E et al, 2010, In vitro bone growth responds to local mechanical strain in three-dimensional polymer scaffolds. *J of Biomechanics*. 43(4); 733-739
- Bagnaninchi P et al, (2010) In-depth imaging and quantification of degenerative changes associated with Achilles ruptured tendons by polarization-sensitive optical coherence tomography. *Phys. Med. Biol.* 55 1-11
- Bhargava S et al, 2008, Tissue engineered buccal mucosa urethroplasty - clinical outcomes. *European Urology* 53, 1263-1271
- De Bank P et al, 2007, Accelerated formation of multicellular 3-D structures by cell-to-cell cross-linking. *Biotechnology and Bioengineering*, 97, 1617-1625.
- Goreham-Voss CM et al, 2010, Cross-shear implementation in sliding-distance-coupled finite element analysis of wear in metal-on-polyethylene total joint arthroplasty, *J of Biomechanics*, 43, 1674-1681
- Green J E F, et al 2009. A mathematical model of liver cell aggregation in vitro. *Bulletin of Mathematical Biology*, 71, 906-30.
- Ingram JH et al, 2007, The use of ultrasonication to aid recellularisation of acellular natural tissue scaffolds for use in ACL reconstruction *Tissue Engineering*, 13, 1561-1572
- Moustafa M et al 2007. A randomised controlled single blind prospective pilot study on the use of autologous keratinocytes on a transfer dressing (Myskin) in the treatment of non-healing diabetic ulcers. *Regenerative Medicine*, 2, 887-902
- Nikolaev NI et al, 2010, A validated model of GAG deposition, cell distribution, and growth of tissue engineered cartilage cultured in a rotating bioreactor', *Biotechnology and Bioengineering*, 105, 842-853
- Thomas RJ et al, 2009, Automated production of a therapeutic clinical human stem cell line. *Biotech Letts*, 31, 1167-1172
- Saleh FA et al, 2010, Regulation of Mesenchymal Stem Cell Activity by Endothelial Cells. *Stem Cells Dev [Early Epub]*
- Stapleton TW et al, 2008, Development & characterisation of an acellular porcine medial meniscus. *Tissue Eng Part A*, 14, 505-518
- Wang L et al 2008, Computational simulation of oxygen diffusion in aortic valve leaflet for tissue engineering applications. *J of Heart Valve Diseases*, 7, 700-9