

# Tissue engineering and cell-based therapies — challenges and progress

A recent symposium provided an insight into the challenges faced in bringing cell therapies to the patient. Joseph Chamberlain reports

**M**an-made biomaterials are used in every part of the body, said Sandra Downes, of the University of Manchester. At the school of materials, work is focused on gaining an understanding of how the body interacts with implanted materials, leading to the development of more biocompatible materials, and on hard-to-heal tissues, such as chronic wounds, and non-healing tissues, such as degenerative cartilage. In the early use of implants a major problem was corrosion, the body being an aqueous, aerated environment — ideal for oxidation of metals. Modern implants are made of biocompatible materials such as ceramics.

To prepare new biomaterials for wound healing, dermal fibroblasts are seeded onto a biocompatible scaffold to form a living tissue. After two weeks, a dermal tissue has formed which can support the migration, proliferation and stratification of an epidermis.

The anterior cruciate ligament has little capacity to repair itself, and damaged ligament can only be reconstructed; it cannot be repaired. Patellar tendon autografting, the standard treatment, suffers from an initial loss of strength, morbidity at the donor site and a lengthy rehabilitation period. An anterior cruciate ligament scaffold can be created with a large number of small braids. The scaffold is mobile when flexed, has a consistent cross sectional shape and is easier to fine-tune to the needs of the patient.

The biomaterials of the future will be dependent on a number of key technology areas, including molecular biology, nanotechnology, proteomics, tissue engineering, biosensors, imaging and informatics said Professor Downes.

## Polymer synthesis

If you want to build a medical device, what material do you use, asked Daniel Anderson, of the Massachusetts Institute of Technology. The early biomaterials were adaptations of existing materials, such as sausage casings for dialysis tubing, and lubricants for breast implants. Recognising that such off-the-shelf products are often inadequate, researchers have turned to preparing materials with properties specifically suited as biomaterials. Rational design is one approach, but often it may be unclear which properties are optimal and an alternative is to adopt a high throughput strategy — synthesising and testing thou-

sands of compounds, getting lucky rather than getting smart. By using microarray plates and combinatorial chemistry, thousands of polymers can be prepared on the nanolitre scale and the same plates tested either for mechanical properties of the new material or against biological material for such properties as cell differentiation. Coupled with intensive development of microscale analytical systems, as many as 1,500 polymer spots can be synthesised and assessed on a single slide. Such arrayed biomaterials constitute a flexible platform for synthesis and testing because polymers can be synthesised on-or-off chip, the chemistry is flexible, and incorporation of drugs and ligands is straightforward.

Dr Anderson went on to explain that there are also many barriers to gene delivery making rational design of a delivery system difficult, although the high throughput approach can be used in this area. In a typical study, 94 amino monomers were combined with 25 diacrylate monomers to produce 2,350 distinct biodegradable polymers with the potential for providing a vehicle for gene delivery. A semi-automated, cell-based screening method tested approximately 1,000 polymers a day and 46 new polymers were identified that could deliver DNA at least as efficiently as can polyethylenimine. Furthermore, these polymers have potential for delivery of other therapeutic agents.

Jason Burdick, of the University of Pennsylvania, explored the possibilities of photocrosslinkable biomaterials. Using ultraviolet light to form polymers is an attractive procedure because polymerisation is well controlled, there is rapid conversion of liquids to solids, solvents and high temperatures are not required, and there is good spatial control over polymerisation.

In one procedure, cells are mixed with a reactive macromer and the mixture exposed to ultraviolet light which results in a gel encapsulating the cells. Alternatively, the monomer can be mixed with a porogen — a compound which can be leached from the product to leave pores in the material — before exposure to light to produce a porous scaffold.

Photopolymerisable biomaterials can be useful for the design of tissue engineering strategies, particularly for orthopaedic tissues said Dr Burdick.

## Stem cells

Stem cells are pluri- or multipotent cells that generate all cell types within an organism or tissue, explained Stephen Minger, King's College London. They are capable of self-renewal ad infinitum and retain normal chromosomal karyotype (that is, they are not tumour cells). Sources include developing organs from the fetus, certain adult tissues and embryonic stem cells derived from the inner cell mass of the very early blastocyst. For successful transplantation, stem and progenitor cells must proliferate for extended periods in culture, the cell phenotype must be stable over time with no loss of pluri- or multipotency, and they must be capable of generating the desired cell types upon differentiation. Stem and progenitor cells or their differentiated progeny must survive implantation, functionally integrate into host tissue (adult), evade immune rejection, and provide long-term therapeutic benefit.

Research in the area of nerve cell regeneration is hampered by the lack of a valid animal model. Nevertheless the King's group claimed the first human embryonic stem cells to be grown in the UK. Such cells will be used to research treatments for Parkinson's disease.

Helen Rippon (Chelsea and Westminster Hospital) described the engineering of lung constructs for *in vitro* screening. The aim was to derive inexhaustible sources of cells and tissues that can be supplied on demand for therapeutic tissue regeneration, repair or replacement. The first target was the lung epithelium. It was shown that stem cells can be converted to type II pneumocytes *in vitro* by directed differentiation using suitable soluble factors, co-culture, and conditioned medium. Dr Rippon stated that *in vitro* work has gone as far as it can go and it is now necessary to demonstrate whether the cells can repair lung tissue *in vivo*. Animal experiments are now under way.

APS

The Academy of Pharmaceutical Sciences is an independent professional body which aims to provide scientific training through conference and seminar programmes, to support focus groups for networking in specialised subject areas, to collaborate with other organisations in Europe and the US and to represent views nationally and internationally.

It works in partnership with the Royal Pharmaceutical Society in a formal agreement to co-develop programmes for scientific events, including the British Pharmaceutical Conference science programmes. Further information can be found on its website at [www.apsgb.org](http://www.apsgb.org).

The meeting, organised by the Academy of Pharmaceutical Sciences, took place at Imperial College London on 19 May

# Tissue engineered clinical products described

Intercytex is a regenerative medicine company, exploiting major advances in developmental biology to fast-track a new generation of cell-based therapies for treatment of tissue and organ failure. UK research director Penny Johnson explained that the human body contained 10–100 trillion cells, all subject to ageing, trauma or disease.

Regenerative medicine envisages the use of appropriate cells either for cell therapy or for tissue engineering. In the development from undifferentiated cells, through differentiated cells and mesenchymal condensates, to rudimentary tissues and organs, at any stage the product can be transplanted to the patient. However managing the whole process inside the body has considerable advantages including a shorter time for the whole process, retained functionality, and reduced cost.

In the area of wound care, ICX-PRO, a product designed actively to stimulate repair in chronic wounds, is in Phase III trials. So far it has been shown to be effective for venous leg ulcers, with diabetic foot ulcers as a second indication.

Skin grafting is a standard treatment for large area burns, lacerations and reconstructive cosmetic surgery. However, harvesting an autologous graft creates another wound, increasing the risk of infection, and causes extensive scarring. ICX-SKN is a true skin substitute being developed in consultation with world leaders in wound care.

Aesthetic medicine is a new and rapidly developing area. ICX-TRC is hair regenera-

tion product about to start Phase II trials. Hair transplant surgery costs up to \$10,000 per procedure and more than one procedure is often required to give an acceptable effect. In the process developed by Intercytex, a small biopsy is cultured and converted by the proprietary step to a product which can be reimplanted. The process is not limited by donor tissue, there is minimal tissue removal (approximately 120 follicles), minimal scarring, minimal pain, bleeding and swelling, and repeat procedures are possible from a single biopsy.

The future development of cell therapy depends on many factors including understanding of customer needs, the regulatory environment, public perception, funding, and manufacturing systems. In the final analysis, concluded Dr Johnson, the existence of a product will depend on what science can do, what the regulations will allow and what the market will bear.

## Burns and chronic wounds

Sheila MacNeil, of the University of Sheffield, described the progress of a successful commercial product — myskin from CellTran, a University of Sheffield spin-out company. This comprises a carrier membrane for the delivery of cultured keratinocytes and is used for patients with extensive burns and chronic wounds.

Extensive case studies were described. Conventional cultured epithelial autografts involve difficult-to-handle fragile sheets and are less than 50 per cent successful. The tim-

ing of production does not always fit the patient's needs. In contrast the mycel procedure incorporates the patient's own cells onto a robust silicone substrate. A thin shave biopsy is taken from the patient and delivered to CellTran.

The CellTran laboratory carries out cell expansion and preparation of membrane discs which are sent by courier within a week to the patient.

Application of the membrane, over a large area if necessary, can be made weekly and the disc removed after three or four days. Typically, complete healing is obtained in six weeks of repeated application of the discs. The procedure has also been used in patients with post-burns complications and in patients with long-standing ulcers resistant to conventional therapy. An advantage of myskin is that it can be applied on an outpatient basis by dressings nurses.

The product works by supplying cells which provide wound cover, stimulating healthy cells in the wound, improving the wound bed and encouraging re-epithelialisation. Tissue engineered skin products offer significant clinical benefit to certain groups of patients and these need to be identified. There is also a need to develop xenobiotic-free culture strategies.

However, reimbursement strategies in UK are not favourable to development of new approaches to wound healing and this is a major problem for commercial investors in this sector, concluded Professor MacNeil.

# Pharmaceuticals and regenerative medicine combined

Drug delivery has long been a driver for clinical and commercial success said Kevin Shakesheff, of the School of Pharmacy, Nottingham University, providing enhanced efficacy, simplified administration, and often improved protection of intellectual property. Delivery of cells as therapeutic agents also provides challenges and opportunities. As for drugs, cell delivery must have clinical applicability, a biodegradable system and some controlled distribution of therapy within the device. Cell systems are generally more fragile, the delivery device becomes the template for tissue formation, and the functionality of the therapeutic effect is highly sensitive to every design feature of the delivery system.

The three-dimensional structure of the product needs to be considered, as has the control of its location. The cells will need to be protected and the extracellular matrix restored at the site. Co-delivery of growth factors is also important.

Regenerative medicine requires cells, from the patient, a donor or a manufacturing source, appropriate architecture, an extracel-

lular matrix provided by the cells or a surface engineered into a scaffold, and growth factors to promote angiogenesis, proliferation and differentiation.

Recent advances in macroporous scaffolds have been brought about by the use of supercritical carbon dioxide in the drug-loading step of manufacture. Injectable scaffolds have also been developed based on temperature sensitive poly(lactic-co-glycolic acid) microparticles. Such biodegradable polymers have a clinical track record and Food and Drug Administration approval for related uses. They are highly porous with defined pore size ranges. There is no solvent or heat generation during scaffold formation *in situ*, and they can be used for protein and cell delivery, said Professor Shakesheff.

## Bone regeneration

Molly Stevens, of Imperial College, London, described some new and successful strategies for musculoskeletal tissue regeneration. The conventional approach is to bring together progenitor cells, a biomimetic scaffold and growth factors in a bioreactor. The harvested

tissue is then transplanted into the patient. A new approach uses the patient's own body as the cell source, the scaffold and the bioreactor. Such an approach has potential advantages.

The role and impact of the healing process is maximal in defining the micro-environment, and there is no need for harvest and in-vitro culture of cells. Immune rejection not an issue and the procedure is adaptable to minimally invasive surgery. There are also few regulatory issues.

The periosteum, an envelope of fibrous connective tissue that is wrapped around the bone, has a proven capacity for regeneration. In studies in rabbits the periosteum around the tibia was used as an *in vivo* bioreactor to grow new bone, mechanically and biologically indistinguishable from the underlying tibia, but not knitted to it. Hence the new bone could be easily removed for transplant into contralateral tibial defects, resulting in complete integration after six weeks with no apparent morbidity at the donor site. Feasibility studies in man are now under way reported Dr Stevens.