Therapeutic Strategies for Tissue Regeneration

by Ian D. Morris and Alan M. Palmer

Gareth Roberts (Novothera Ltd, London, UK) reviewed the path of regenerative medicines from promise to marketed product. The approach of regenerative medicines sits within the context of the body healing itself. This can occur by 1) replacing damaged tissue (e.g., organ transplantation), 2) by tissue repair (e.g., adding new cells to an organ) and 3) tissue regeneration using factors to stimulate cell renewal. Organ transplants are now a well-established treatment, but patients have to endure life-long immunosuppressive treatment, and demand outstrips the supply of fresh organs. Dr. Roberts pointed out that a new organ is needed every 18 minutes. The approaches of tissue repair and tissue renewal offer the possibility of not just reducing the demand for organ replacement but also of moving into new vistas for regenerative medicine, such as diabetes and Alzheimer’s disease.

The concept of regenerative medicine has become increasingly prominent over the last few years, with a great deal of attention focused on rebuilding tissue via the addition of new cells (i.e., cell therapy). This has been greatly stimulated by the use of stem cells derived from both embryos and adult tissues, such as fat and bone marrow. Novothera Ltd was spun out from the Tissue Engineering & Regenerative Medicine Centre at Imperial College (London, UK) to specialize in biomaterials and regenerative therapies. Their core technologies include bioactive material platforms, some of which use soluble glasses and Solgels. Adult and embryonic stem cells (both murine and human) and engineered tissue, e.g., osteoprogenitor cells can be grown on these scaffolds.

Regenerative strategies for bone

Dr. Roberts went on to introduce Novothera, who aim to apply BioGlass approaches to therapy in the area of wound healing/bone repair. An example of its utility is shown by its ability to mimic trabecular bone. In collaboration with Dr. P.D. Lee, Department of Materials, Imperial College and using a bioactive glass foam scaffold, they were able to effectively improve the outcome of an individual who sustained a compound fracture of the left orbito-zygomatic complex in a high-speed road traffic accident.

While some approaches to tissue regeneration use exogenous stem
cells, others utilize those already in place and capitalize upon their presence. However, this is an inefficient method of delivering very complex therapeutics. The tissue architecture may be destroyed or absent and some guidance is required for the growing cells if a normal tissue is to result as Prof. Kevin Shakesheff (Tissue engineering group, University of Nottingham, UK) went on to explain to the audience. Tissue engineering would be more effective if the natural self-assembly process could be recapitulated. Engineering of the scaffolds can be used to define the pore structure and subdivision of the threedimensional space as well as the surface properties mimicking the extracellular matrix that interfaces between cells and the scaffold. Additionally, the scaffold may be manufactured to include soluble growth factors to give various concentrations and release profiles. The biomechanical properties are also an important consideration, as the transmission or negation of mechanical forces influence the final tissue architecture. The Nottingham group has established development programs in liver, bone and cartilage to design novel scaffolds for minimally invasive delivery of cell and protein therapeutics. One of the novel technologies described was the production of bioactive scaffolds by solvent free low temperature technology. Using supercritical fluid mixing, this technology overcomes the problem that many proteins are unstable and lose biological activity during manufacture. If liquid CO$_2$ is used as the vehicle, bioactivity is retained. Supercritical CO$_2$ permeates solid materials and acts as plasticizers for polymers. The process can produce polymers of various matrix sizes in particulate form when the supercritical fluid is released through an orifice or as a shaped solid if released into a mold.

Release of the protein can be manipulated by the use of different polymers but is usually slower than the degradation of the polymer. Current in vivo experiments in bone regeneration with BMP-2 encapsulated with PLA indicted a superior delivery profile compared with release from a collagen sponge. Surprisingly, this technology can also be used to incorporate live cells into scaffolds. Cells survive maximum pressures (about 70 bar) for up to 1 minute and decompression rates prolonged to 4 minutes. Another novel technology under development is colloid aggregation scaffolds, which are used for space filling in vivo by minimally invasive delivery, so delivering therapeutic bioactive molecules or cells to the regeneration site. The polymers, microparticles and cells are mixed with a cross linker and injected into the cavity. The cross linker is activated as the mixture warms to body temperature producing a fused macroporous scaffold. When tested in bone, the implant formed within 15 minutes and is able to hold its shape over a prolonged period of time. Eventually, the polymer biodegrades and is replaced by mineralized bone. Professor Shakesheff concluded that the roles of intelligently designed scaffolds are essential in tissue engineering to ensure that therapeutic cells or proteins are delivered to the right place at the right time.

Dr. Terry Sachlos (TEOX Ltd, Oxford, UK) addressed some of the limitations of current treatments of bone disease and how unmet needs would rise as the incidence of fractures increases in the years ahead. Currently, many bone replacements use autografts of bone harvested from the iliac crest of the pelvis, but their ubiquity is limited by the paucity of the material that can be obtained. Cadaver allografts and biomaterial scaffolds have been used with limited success, so it seems that tissue engineering approaches would be the most promising way forward. As reviewed in Prof. Shakesheff’s presentation, sophisticated engineered scaffolds are being developed; however, their success maybe limited as they do not possess blood vessels to deliver blood nutrients for the support and survival of cells deep inside the scaffold. Additionally, the physical shape of conventional scaffolds do not reflect the in situ injury, producing problems of tissue incorporation as well as cosmetic considerations. Dr. Sachlos showed clearly how the Haversian system containing capillaries and nerves was central to bone function. TEOX has developed a technology based on three-dimensional printing that incorporates micro-channels inside collagen-based, shaped scaffolds which allow for circulation of nutrient- and oxygen-enriched extracellular fluid. The biomimetic technology uses collagen membranes onto which hydroxyapatite is precipitated producing a structure which reflects collagen fibers reinforced with mineral. The prototyping manufacture used is three-dimensional printing with wax overtyping that is dissolved to form the mold for the collagen mixture; this material is thus assembled into a scaffold with a channel size of 4–220 microns. Eventually, the goal is to promote angiogenesis within the channels. An important step for therapeutic use is that the implant shape can be customized to each patient by interfacing data from medical imaging NMR scans with the software controlling the shape of the printed mold. The use of digital technology to fabricate patient tailored scaffolds is an attractive substitute in low-load-bearing reconstruction. The company’s first product is an implant which is shaped for the jawbone, and later products will include bone for hip and knee replacement, and vertebral implants. Eventually, the shaped scaffolds will be used to produce viable cell implants for heart, blood vessels and liver.

Regenerative strategies for skin disorders

A human fibroblast three-dimensional culture that has been developed for the treatment of chronic wounds was described by Jonathan Mansbridge (Smith and Nephew Wound Management, La Jolla, California, USA). The cells are grown on a biodegradable scaffolds which
encourages the secretion of matrix proteins and growth factors. During production a lactate/glycolate copolymer is formed into biodegradable knitted scaffolds and seeded with allogenic cells from a single individual stored in a Master cell bank. The cells in this bioreactor are grown for several days, and the product harvested and cryopreserved until used. This preparation, Dermagraft™, has been used successfully for some time and significantly advances wound closure. The cells in the preparation colonize the wound bed, secrete cytokines, including angiogenic growth factors, and provide a substrate for the patient’s keratinocytes as well as promoting the invasion of inflammatory cells. Dr. Mansbridge suggested that chronic wounds may arise because of an inappropriate senescence response of endogenous fibroblasts to injury, leading to bacterial colonization and failure of the wound to heal. IL-8 secreted from Dermagraft overcomes this deficit as it attracts and activates neutrophils most likely through the CXCR receptors. Further studies using microarrays revealed the complexity of the response of the cells in Dermagraft, 22 genes were unregulated more than five fold, one of the most dramatic being IL-8, substantiating his hypotheses of the reasons for the lack of healing in chronic wounds. Dermagraft action was therefore multifaceted and included neutrophil and monocyte immigration, tissue granulation and re-epithelialization.

The role of substantial R&D investment to produce a successful product was emphasized by Dr. Mansbridge. This unique product required novel solutions. The master bank was expanded carefully to ensure the minimum number of passages before establishing a stock from which to grow the product. Safety testing was rigorous and excluded tumorigenicity as well as microbiological contamination. Scaffold consistency was important to the stability and strength of the dressing, both during in vitro production and when used therapeutically. During production the bioreactors are coupled together in a closed system which maintained asepsis of the final product, especially as the procedure used part of the bioreactor in the packaging. The final preparation is cryopreserved and only distributed after sterility testing. Interestingly, on thawing for use the viable cells mount a cellular stress response, and 24 hours later substantial gene expression is induced which Dr. Mansbridge indicated most likely contributes towards the efficiency of Dermagraft™.

Regeneration strategies must also take account of the appearance of the restored tissue. Mark Ferguson (Renovo Ltd, Manchester, UK) clearly demonstrated that endogenous processes which occur after traumatic injuries such as burns can leave the patient with substantial scarring, restriction of movement and major psychological injury. It has been estimated that 42 million people per year have unwanted scarring, and currently there are no protocols marketed for its prevention and reduction. It has been long been recognized that fetal wounds heal without scarring and the academic work of Prof. Ferguson identified that growth factor signaling was different in adult and fetal wounds. In particular, the expression of tumor growth factor β3 (TGF-β3) was high in fetal scar-free healing while the isoform TGF-β1 was high in adult scarring wounds. Importantly, addition of TGF-β3 to adult wounds reduced or eliminated scarring whereas deletion of the gene caused enhanced scarring. Histological examination of TGF-β3 treated wounds indicated that it was a potent skin morphogen that promoted the renewal of normal tissue architecture. In vitro, dermal cell fibroblasts stimulated by TGF-β3 through a Ras-GTPase pathway were found to migrate faster and more randomly and were accompanied by increased cell survival as well as the laying down of collagen in a basket weave configuration.

Renovo Ltd has used this profile to develop recombinant TGF-β3 to promote scar-free healing. The product, Juvista™, has completed several phase 1 and 2 safety and efficacy studies. In one phase 2, randomized, double-blind, patient-controlled trial, 42 subjects received experimental skin incisions, and the drug was applied to the tissue surrounding the wound by injection. Clear cosmetic improvement was seen as early as 2 months, and by 6 months the wound site was not visible. A more demanding study was also described in young males (poor healers) with wounds under tension, and treatment produce the same beneficial result. Examination of tissue collagen architecture at 6 months showed an improvement by 100% in the treated group. Ongoing studies include Juvista treatment in skin donor grafts, head and neck nevi, and wounds after breast augmentation.

Wound healing is a multifactorial process and it seems unlikely that the only druggable target will be TGF-β3. The Renovo research team have embarked upon examination of the transcriptome of healing wounds. As expected, there are significant differences between TGF-β3 treated and control wounds. Close analysis revealed two population groups that can be roughly divided into responders and nonresponders. In responders gene expressions were significantly clustered suggesting the activation of several different biological processes. An interesting development from their studies was a comparison of the expression profile in certain Caribbean populations predisposed to keloid formation. The gene profiles were different in these individuals, offering the possibility of developing more personalized treatments. Professor Ferguson finished his talk by drawing attention of the audience to Lucy Grealy’s book ‘Autobiography of a face.’ The quotation which follows undoubtedly convinced the audience of the need to develop treatments that are acceptable to both the clinician and the patient. “I spent 5 years of my life being treated for cancer, but since then I’ve spent 15 years being treated for nothing other than looking.
different from everyone else. It was pain from that, from feeling ugly, that I always view as the great tragedy of my life. The fact I had cancer seemed minor in comparison.”

**Regenerative strategies for CNS disorders**

Stem cells are multipotent and clearly essential for the process of development. As mentioned earlier, they offer great potential in the process of tissue regeneration following injury. Dr. John Sinden (ReNeuron Ltd, Guilford, UK) described the use of stem cell therapies for tissue regeneration following brain injury. Transplantation of neural stem cells into the brain is a rapidly emerging novel approach to the treatment of chronic neurological diseases, such as Parkinson’s disease and Huntington’s disease.

Dr. Sinden pointed out that stem cells have different potencies, they can be: 1) totipotent and thus capable of recreating an entire organism; 2) pluripotent and give rise to cell types within tissues of the origin germ layer (these are derived from somatic tissues in the developing or adult organism, e.g., bone marrow, blood, muscle, liver, brain, pancreas, retina); or 3) multipotent such as somatic stem cells which have a capacity for self-renewal, without being cancerous. Examples include: hematopoietic stem cells (blood lineage), mesenchymal stem cells (connective tissues), liver oval cells, muscle satellite cells and neural stem cells.

ReNeuron have established a prototype neural stem cell line (MHP36), which is a stable diploid cell line from H2kbtsA58 transgenic mouse E14 hippocampal neuroepithelium. These cells have been shown to migrate preferentially to lesion damage and demonstrate site-specific differentiation into neurons and glia in vitro and in vivo. In several laboratories worldwide this approach has been shown to be effective in global ischemia, spinal cord injury, stroke and aging, lesion models of memory loss and Huntington’s disease and traumatic brain injury.

For clinical application, safety and efficacy of defined, stable and scaleable, quality-assured cell lines produced under GMP conditions are required. To this end, a human neural stem cell line, CTX0E03, was derived from human somatic stem cells (from 12-week fetal cortex) following genetic modification with a conditional immortalizing gene, c-mycER™. This transgene generates a fusion protein that stimulates cell proliferation in the presence of a synthetic drug 4-hydroxy tamoxifen (4-OHT). The cell line is clonal, expands rapidly in culture (doubling time 50–60 h) and has a normal human karyotype (46 XY). In the absence of growth factors and 4-OHT the cells undergo growth arrest and differentiate into neurons and astrocytes. Transplantation of CTX0E03 in a rat model of stroke caused statistically significant improvements in sensorimotor function and motor asymmetry tests at 6–12 weeks post grafting. In addition, cell migration and long-term survival in vivo was not associated with significant cell proliferation. These data indicate that CTX0E03 has the appropriate biological and manufacturing characteristics necessary for development as a therapeutic cell line. It is now moving towards phase I studies to evaluate safety of the implantation technique.

Another cell line that meets ReNeuron’s criteria for clinical development is STR0C05, a cell line from 12-week fetal striatum which is in late preclinical development for Huntington’s disease. Potential therapy for Parkinson’s disease is seen in their cell line, REN-004, which produces dopaminergic neurons. Efficacy has not yet been demonstrated in experimental animals.

Dr. Greg Hamilton (Guilford Pharmaceuticals Inc, Baltimore, USA) described the utility of neuroimmunophilin ligands in the restoration of function in neurodegenerative disease. In the early 1990s, reports from the laboratories of Drs. Solomon Snyder and Bruce Gold suggested that the immunosuppressant drug FK-506 possessed neuroprotective and neuroregenerative properties. In PC12 cells, FK-506 was found to enhance the ability of nerve growth factor to promote neurite outgrowth and enhanced recovery of sciatic nerves in rats following surgical lesioning. FK-506 exerts its immunosuppressant effects by binding to the 12 kD FK506-binding protein (FKBP12) and subsequent inhibition of the phosphatase, calcineurin, by the FKBP-FK506 complex. Subsequently, scientists at Guilford Pharmaceuticals demonstrated that the neuroregenerative effects resided in the FKBP binding domain portion of FK506, and that nonimmunosuppressant analogs of FK506 could be designed that retained the neuroprotective and neuroregenerative effects of FK-506.

Dr. Stuart Schreiber, who elucidated the immunosuppressant mechanism of FK-506 15 years ago, coined the term “immunophilins” to denote proteins such as FKBP12 and cyclophilin A that bind immunosuppressant drugs and mediate their actions. Immunophilins such as FKBP12 and cyclosporin are highly expressed in the mammalian brain, and these CNS-residing proteins are the neuroimmunophilins. Several structural classes of small-molecule FKBP ligands have been synthesized and studied.

Guilford Pharmaceuticals synthesized the first nonimmunosuppressant, orally bioavailable drugs with affinity for FKBP. The prototypic example is GPI-1046, which promotes neurite outgrowth from dorsal root ganglion cultures in vitro. GPI 1046 and numerous other compounds have been shown to significantly restore striatal dopaminergic nerve function following lesioning of mouse brain by the neurotoxin MPTP. These effects are observed both when the drugs are given concomitantly with the toxin (suggesting a protective effect) and
when given after toxin-induced lesioning (suggesting a regenerative component of action). These results were subsequently replicated in primates.

Administration of neuroimmunophilin ligands may also be used as adjuncts to prostate surgery, as they may protect the penile cavernous nerve from traumatic damage. GPI 1046 provides neuroprotection for penile innervation from degeneration following cavernous nerve crush injury in rats. Intracavernosal pressure responses to electrostimulation of injured and intact cavernous nerves were recorded for each animal at 24 hours or 7 days post surgery. Animals treated with either FK506 or GPI 1046, as compared to untreated animals, showed nearly complete protection of NOS neurons following the lesion, and maintenance of intracavernosal nerve pressure.

Guilford Pharmaceuticals have now developed second-generation neuroimmunophilin ligands, and GPI 1485 is the prototypic example. Phase I results indicate GPI 1485 is safe and well tolerated up to 6000 mg and is orally bioavailable (>70%). It is currently in two phase II clinical trials for Parkinson’s disease and erectile dysfunction following radical prostatectomy. Although an initial trial in Parkinson’s disease was not long enough to detect disease modification, a new study is designed to detect slowing or reversal of disease progression using 200 patients with mild to moderate idiopathic Parkinson’s disease. Instead of using UPDRS motor “off” as a primary outcome measure, this study will use changes in brain structure, assessed using SPECT at 2 years as the primary outcome measure.

An entirely different approach to tissue regeneration was introduced by Dr. Lee L. Rubin (Curis Inc., Cambridge, Massachusetts, USA) taking advantage of the understanding that tissue development in the embryo is predominantly controlled by a small number of powerful regulatory pathways. One of these is the signaling pathway activated by the three peptide ligands of the hedgehog pathway. The Hedgehog (Hh) family of proteins was originally discovered in Drosophila and in mammals comprise:

- Indian Hh, which is necessary for the development of cartilage and bone
- Sonic Hh, which is necessary for the development of the central nervous system
- Desert Hh, which is important in the development of peripheral nerves.

Activity of the Hh pathway is a major regulator of different tissues in organisms ranging from Drosophila to human. In mammals in the absence of adequate Hh activity, birth defects occur including a disorder that leads to the development of a single eye (Cyclopia). As the Hh pathway controls processes that balance proliferation and differentiation it has also been implicated in cancer, which reflects the efficacy of Hh antagonists to inhibit growth of medulloblastoma cells, both in vitro and in vivo. Activation of hedgehog protein has been linked to cancers of the esophagus, lung and stomach as well as basal cell carcinoma and pancreatic adenocarcinoma.

In adult rodents, Ptc-1 (the Hh receptor), Smo (an Hh pathway signaling protein) and Gli-1 (a transcription factor activated by Hh signaling), are expressed in relatively high concentrations in regions where neural precursors reside (e.g., around ventricles). Hh overexpression stimulates the proliferation of stem cells in the adult brain and increases their neuronal differentiation. There is evidence to suggest that the Hh pathway continues to affect the adult CNS, which motivated Curis to identify and optimize a family of small-molecule Hh agonists. The Hh agonists have sub-nM EC₅₀, are orally available and permeate the central nervous tissue. These Hh agonists control cell proliferation and differentiation in a normal cell type-specific way; thus, Hh agonists stimulate proliferation of cerebellar granule neuron progenitors. Hh agonists also induce motor neuron differentiation, in embryonic spinal cord explants, and stimulate proliferation of adult neural stem cells. Oral administration of Hh agonists also increases proliferation in mouse subventricular zone and hippocampus, which has led to the hope that such activators of neurogenesis will be of value in CNS disorders such as depression, Alzheimer’s disease, Parkinson’s disease and stroke.

Recent data suggest that various chronic antidepressant treatments increase adult hippocampal neurogenesis that mediates the behavioral effects. Dr. Rubin described how dosing with Hh agonist has clear (but modest) efficacy in a model of anxiety/depression, substantiating the idea that small-molecule Hh agonists may be useful therapeutics for treating neural disorders. Curis have established an alliance with Wyeth Pharmaceuticals to develop neurogenic compounds, with stroke as the lead indication, especially as Hh agonists display neuroprotective efficacy in addition to their ability to regenerate injured brain tissue.

Since Hh agonists induce motor neuron differentiation from mouse ES cells, they may well have utility in the treatment of motor disorders. Spinal muscular atrophy (SMA), the second leading genetic cause of death among infants, is a motor neuron disease with an incidence of 1 in every 6000 births; the prevalence is about 20,000 in the United States. Curis has developed an effective drug discovery screen using motor neurons derived from ES cells differentiated with a combination of retinoic acid and an Hh agonist. By employing a MN-specific promoter and FACS isolation of cells, they transplanted engineered cells into embryonic chick spinal cord and found Hh agonists induce motor neurons projecting into muscle of developing embryos. Curis is in a unique position to carry out screens in motor neurons.
neurons for compounds that will elevate SMN protein levels and may eventually lead to treatments.

Concluding comments

Regenerative medicine research has taken us to the cusp of some exciting new therapies that have the potential to complement, and possibly replace, many of the current mainstream pharmaceutical treatments. The meeting drew upon the experiences of scientists in several areas where tissue regeneration provides the ideal solution to problems that are not amenable to conventional therapeutic approaches.

A webcast of this meeting can be viewed free-of-charge at http://webcasts.prous.com/SMR_JUNE2005/ or at http://regenerativemedicines.com

Ian D. Morris and Alan M. Palmer are Conference Organizers and Committee Members of the Society for Medicines Research. The SMR Committee organizes conferences on behalf of the Society for Medicines Research four times a year. SMR symposia focus on research related to the discovery and development of new medicines and are usually held in London. Details about forthcoming meetings can be obtained from the SMR web site: www.smr.org.uk or from: SMR Secretariat (secretariat@smr.org.uk).

INTERMUNE UPDATES
STATUS OF IPF PROGRAMS FOR PIRFENIDONE AND INTERFERON GAMMA-1B

InterMune has updated the progress of its two phase III programs evaluating two separate compounds for the treatment of idiopathic pulmonary fibrosis (IPF).

The randomized, double-blind, placebo-controlled, multinational phase III INSPIRE trial of Actimmune® (interferon gamma-1b) has achieved strong enrollment to date, with 600 patients expected to be enrolled across 80 sites in North America and Europe by the end of 2005. The trial is scheduled to conclude 2 years after the 600th patient enrolls, or around the end of 2007. The INSPIRE trial aims to determine if interferon gamma-1b decreases mortality in IPF patients. A blinded, prespecified sample size reevaluation was recently conducted to determine if the observed overall mortality rate was consistent with the mortality assumptions underlying the trial design. At this early point in the trial, the overall mortality rate observed was somewhat lower than projections. To increase the likelihood of reaching the total number of events upon which the INSPIRE trial is powered by the time it is scheduled to conclude around the end of 2007, the company has decided to enroll approximately 200 additional patients in the trial. InterMune anticipates enrolling the 800th patient in the first half of 2006. Enrolling additional patients will not increase the trial duration. Topline data are expected in early 2008. InterMune markets Actimmune® for the treatment of chronic granulomatous disease and severe, malignant osteopetrosis. In addition to the INSPIRE trial, interferon gamma-1b is also being studied in the phase III GRACES trial in ovarian cancer.

InterMune has finalized the phase III program design of its second pulmonology program, evaluating pirfenidone for IPF. The program will involve approximately 550 patients in two separate multinational phase III trials. Lung function, as measured by changes in forced vital capacity, will be the primary endpoint. The program is expected to begin in the first half of 2006, with trials to run concurrently. Pirfenidone is an orally active, small molecule that shows a wide range of biologic activity. In vitro evidence has shown that pirfenidone inhibits collagen synthesis, downregulates profibrotic cytokines and decreases fibroblast proliferation. Data from four phase II trials in over 250 patients suggest that pirfenidone may impact lung function and disease progression in patients with IPF. In 2004, pirfenidone received U.S. and E.U. orphan drug designations for the treatment of IPF. InterMune has worldwide rights, excluding Japan, Korea and Taiwan, to develop and commercialize pirfenidone for all fibrotic diseases.