

## **Advanced Manufacturing Systems for Clinical Grade Stem Cells**

*(Co –Sponsored by ISCT-Europe)*

There has been relatively little progress in the development of new culture technologies for the manufacture of clinically useful progenitor cells and most efforts, including those in small-scale GMP facilities, rely on laboratory-scale methods with little improvement over earlier efforts. There is a strong possibility that progress in delivering therapy to patients will be hampered by a poor ability to produce massive numbers of stem cells, at relatively low cost and with a high degree of uniformity. In the event that therapeutic applications are successful, it is likely that there will be a significant gap between demand and supply. Current approaches, which often rely on serum-containing media and relatively low volume methods, will not be effective and methods for testing MSC preparations rely on panels of surface antigens which may be non-specific. This symposium will focus on current approaches to the development of advanced manufacturing systems for mesenchymal stromal cells (MSCs). These cells are selected because of their applicability in a broad range of diseases, their allogeneic use and the considerable profile of safety that applies. Combinatorial approaches to the selection of new methods of culture involving growth factor supplements to modulate and optimise growth kinetics and differentiation will be discussed. Physical expansion systems will be the focus of some discussion, including advanced robotics, high density flask systems and microcarriers. The development of methods for in-line testing of cells and media will also be addressed. These will include automated FACS to determine cell phenotype, the use of Raman spectroscopy to monitor media composition and rapid analysis of protein profiles to determine heterogeneity. The regulatory aspects of stem cell manufacturing will be a discussed. The issues of GMP compliance and the application of quality systems will be addressed. These discussions will attract interest from stem cell biologists, bioprocess engineers, clinicians, regulatory specialists and industry representatives.

## **Biomaterials and Engineered Constructs - Outcomes in Medicine/Existent Surgery**

Biomaterials development resulting from extensive basic research has to be translated in the clinical setting to determine their suitability or their shortcomings in human applications. Translational research will involve the investigation of biomaterials that have been developed under optimal laboratory conditions, but have to be utilized under complex clinical and surgical pathological states. The Biomaterials & Engineered Constructs- Outcomes in Medicine/ Existent Surgery (BECOMES) Group focuses on the translation outcomes of biomaterials and generated tissues in clinical and contemporary surgical applications. The group focuses also on the better understanding of the clinical pathology and relating the difficulties experienced by the clinicians and surgeons in their practise to the tissue engineering community. The group aims to highlight to Researchers in Tissue Engineering the coexistence of conditions (co-morbidities) that will affect or alter the primarily intended functioning of the original biomaterial or engineered tissue. The foremost intention of the group is to expose the Basic Science Research Community in Tissue Engineering with the ground realities in patient pathology and the difficulties experienced by the Medical Practitioners and Surgeons. This exposure is intended to help in the translational research and evaluation and implementation of Tissue Engineering Technologies in Clinical practice.

The symposia intention of the BECOMES Group is 4 fold:

- (a) **Outcomes of Biomaterials in Contemporary Clinical Applications:** Presentations will be invited from researchers who have applied biomaterials or engineered constructs in the clinical practise. These presentations are intended to showcase the ease or difficulties in the application of these materials in humans. The usage of these materials, their outcomes and their shortcomings will be presented and technical improvements that are desired will be presented to researchers in the area of Tissue Engineering.
- (b) **Identification of Clinical states demanding Regenerative Medicine:** The second area of presentation will be exploring the clinical states that require biomaterials or engineered tissues. Presentations will be made to expose clinical conditions and the present state of palliative therapies that are offered to the patients. These presentations are intended to expose tissue and clinical states that have not part of the frontline research in tissue engineering, however the demand of tissue is these area is so dire that millions of Euros are being spent in the management of these patients with no optimal solutions in sight.

- (c) **Focus on Paediatric organ loss:** The focus of the tissue engineering research is mainly on the adult populations and the conditions encountered later in life. There is even a much larger shortage of organs in the newborns, infants and the childhood age group that the tissue engineering research community is not aware about. Paediatric organ shortages are further complicated by donor mismatches (for example if an adult liver donor is found for a newborn who requires a liver transplant- it is almost impossible to fit an adult liver in the child).
- (d) **Biomaterial research in in-vivo animal models:** Presentations will be also done on in-vivo animal models to explain the working of biomaterials or generated tissues in these experimental studies. These presentations will be important for the clinicians and surgeons to understand the development and the present stage of research in animal experiments and the future clinical applications.

### Biophysical Cues and Cell Behavior

This symposium will focus on the role of biophysical cues in directing cell behaviors. Specifically, the role of topographic cues, compliance of the cell substratum and the galvanic properties of the cellular microenvironment will be examined in the context of stem cell biology, wound healing and prosthetic design. Each of these attributes of the cellular microenvironment have emerged as key regulators of fundamental cell behaviours including adhesion, orientation, migration, proliferation and differentiation. The molecular mechanisms underlying their effects are being intensively studied. In aggregate, these parameters have immediate relevance to evolving strategies in cell, stem cell and tissue engineering and the design and fabrication of implantable prosthetics... Additionally; the failure to incorporate these cues in cell culture plasticware may partially explain why so many in vitro studies fail to successfully translate into the clinic. Finally, the elucidation of the molecular mechanisms underlying the cellular consequences of biophysical cues may point to new therapeutic frontiers.

### Cardiovascular Tissue Engineering: From Bench to Bedside

Cardiovascular disease is the most frequent cause of mortality in the Western societies. Despite significant advances in cardiovascular medicine in the last decades, surgical intervention for the treatment of occluded vessels or stenotic and regurgitant heart valves is far from perfect. Conventional therapies for heart valve dysfunction are associated with the limited durability of the bioprostheses, the need for anticoagulation with the mechanical valves and the limited availability and potential immunogenicity of the homograft valves. On the other hand, conventional angioplasty techniques are still hindered by late thrombosis events and in-stent restenosis, while synthetic grafts have proven to evoke thrombogenic responses in small-diameter applications. Autologous grafts remain the gold standard for both heart valve and small-diameter vessel replacement, since they will retain viability and regenerate. In many patients, however, autologous tissue is not available, and even if it is available, this is not an ideal solution. Tissue engineering approaches to cardiovascular tissue replacement have made considerable advances over recent years and it is likely that such applications will realize clinical success in the near future. Research in this area has been driven by the inadequacy of the currently available cardiovascular prostheses for younger patients, who require multiple reoperations as they grow and develop. Tissue engineering has the potential to provide grafts capable of growing and repairing, and could improve outcomes for patients of all ages. Owing to the function and physical environment of cardiovascular tissues, the development of tissue-engineered replacements requires that the biomechanical properties of the graft are given priority over its biological properties, in order for the graft to be functional at the time of implantation. As such, considerable effort has been directed towards the development of tissue engineering scaffolds with adequate mechanical integrity, able to withstand the demanding haemodynamic environment *in vivo*. Novel approaches also include the functionalisation of these scaffolds with various peptides with a view to tethering endogenous endothelial cells *in vivo*. Although more translationally challenging, several *in vitro* tissue engineering strategies have also been developed. These are focused on seeding appropriate stromal cells or stem cells on a variety of scaffolds, including synthetic polymers, decellularised tissues, reconstituted proteins and self-assembled matrices, and subjecting the cell-scaffold constructs to dynamic physical conditioning protocols *in vitro*, with a view to enhancing graft functionality prior to implantation. Major areas of debate

in the field of cardiovascular tissue engineering include the appropriate source of and type of scaffold, whereas the type and appropriate amount of physical conditioning needed to improve tissue formation and regeneration *in vitro* remains speculative. The aim of this symposium is to bring together the leading expertise in the field of cardiovascular tissue engineering, focusing on the different aspects involved and the advantages and disadvantages of the different approaches that have been adopted by researchers in the field. In particular, this symposium will be focussed on studies that have reached the pre-clinical/clinical stages of development of tissue-engineered heart valves and vascular grafts, with a view to determining how close the field lies to significant clinical translation. This symposium will also provide an insight into what lies on the horizon for cardiovascular tissue engineering including areas of future research focus.

### **Cells, Matrix and Mechanobiology**

The success of tissue engineering will be dependent on the interactions between the cells, the matrix and mechanical force. The extracellular matrix plays a crucial role in tissue function, dictating its physical and mechanical properties, maintaining the spatial arrangement of the cells that reside within it and mediating the complex crosstalk that exists between the cells, the matrix and external forces. The matrix controls cell size, shape, motility and alignment through its three-dimensional architecture and focal adhesion organisation. Equally, the cells influence the matrix by applying traction forces and by synthesising as well as degrading matrix components. In addition, the interaction between specific recognition sequences displayed on extracellular matrix components and cell membrane receptors is responsible for triggering a variety of specific cellular functions. It has become increasingly clear that the mechanical environment is equally important as, and synergistic with, the chemical environment in directing cell behaviour and fate. Mechanotransduction signalling pathways induced in response to mechanical force are essential for the maintenance and function of tissues and can be exploited for tissue engineering applications to direct cellular function and create the desired end product. Tissues throughout the body are exposed to different degrees and different forms of mechanical force, however, the amount of mechanical force perceived by the cells within the tissue is unlikely to equal that of the applied force, due to stress shielding and strain transfer. In addition, many tissues display anisotropy, with different micro- and gross-mechanical responses in the circumferential and radial directions, which has a major impact on tissue biomechanics, strain transfer and subsequent cellular responses. Load-bearing soft tissues such as heart valves and tendons, which consist of a network of fibrous protein (predominantly collagen and elastin), embedded in a gel of proteoglycans, glycosaminoglycans and glycoproteins exhibit anisotropic, non-linear, visco-elastic behaviour, coupled to the organisation of the collagen fibres. Studies have begun to dissect the mechanisms of tissue biomechanics and subsequent cellular responses and the role played by collagen, however little is known about the influence of other matrix components on these mechanisms. Investigations in native tissues, engineered scaffolds, model culture systems, and computational models are rapidly expanding our understanding of mechanobiology and this knowledge is now becoming incorporated into novel scaffold and bioreactor design. This symposium aims to examine the signalling pathways activated in response to physiologically relevant mechanical force through the interaction of cells with different matrix components. The symposium also aims to provide a better understanding of tissue biomechanics and a more realistic estimation of the forces perceived by cells within tissues by examining and defining the role of individual matrix components and investigating mechanisms of stress shielding and strain transfer through the matrix. Successful tissue engineering requires a comprehensive understanding of mechanobiology and in particular the loading conditions experienced by the cells under physiological conditions, in order to establish how this controls cellular functions. Elucidation of mechanotransduction pathways and a clearer knowledge of the relationships between physiologically representative loading conditions and cell strains, should provide useful information for tissue engineering and regenerative applications as well as further insight into mechanisms involved in disease processes.

Presentations will be selected from submitted abstracts in the following areas:

- (i) Receptor mediated interaction of cells with extracellular matrix components
- (ii) Mechanotransduction/signalling pathways
- (iii) Tissue biomechanics

## Cellular Differentiation in Tissue Development

### Computational Modelling in Tissue Engineering

Computational modelling is an important tool in tissue engineering, as it contributes to various aspects, essential for our fundamental understanding of underlying mechanisms, as well as for the development of new technologies. The symposium wants to focus on these contributions, and will cover the following topics:

- Computational modelling of cell and tissue dynamics, relevant for tissue engineering
- Computational models to quantify mass transport in tissue engineering constructs
- Use of computational techniques to optimise scaffold design
- Use of computational techniques to optimise bioreactor design

The symposium wants to demonstrate that, by combining computational analysis with (in vitro or in vivo) experiments, new possibilities are being created both in terms of fundamental understanding as well as applications. Finally, as computational models in tissue engineering may involve multiphysics and multiscale aspects, the development of such models may also be challenging from a methodological point of view. Although the symposium will be focused on the application to and added value for tissue engineering, methodological aspects may be covered as well.

### Endochondral Ossification

The fields of tissue engineering and regenerative medicine (TERM) have improved noticeably in recent years with new developments and progression in bone and cartilage repair too numerous to discuss here. Despite this, very few clinical studies have been performed with regard to tissue engineered bone with varying success with even fewer studies at the bone-cartilage interface. There is currently a huge deficit in the available options for the millions of bone and joint replacement procedures performed every year worldwide. Tissue engineering of bone has, since its inception, focused on the generation of bone via the direct differentiation of mesenchymal stem cells to bone forming cells, a process called intramembranous ossification. Normally, in vivo, this process is characterised by an increase in vascularity of the existing connective tissue present prompting invasion of mesenchymal cells into the region. These cells then differentiate along the osteogenic route to become osteoblasts which will lay down the organic component of bone, called osteoid. This process however, normally occurs only in a small proportion of special bones within the body such as the skull. Most bones of the body however, are formed via a process known as endochondral ossification, whereby a cartilage template is first generated, which is subsequently invaded by blood vessels (stimulated by this cartilage tissue) and mineralised to form bone. If one considers that one of the main reasons for failure of a tissue engineered construct is that of core necrosis caused by hypoxia and lack of nutrient delivery and waste removal perhaps a complete rethink of the approach is required. It has become very clear that to successfully generate viable tissue engineered bone vascularisation must be accounted for. There is however perhaps a much more logical approach to form bone. Why not induce bone formation by endochondral ossification? In this scenario a construct of chondrogenically primed MSCs or perhaps even a MSC derived cartilage template would be implanted and allowed to progress further along the endochondral ossification route to form bone naturally. This approach has several advantages. Firstly MSCs are known to progress along the endochondral ossification route naturally becoming hypertrophic as occurs very often in cartilage tissue engineering approaches. Secondly chondrocytes usually reside in an environment of very low oxygen tension. Placing an unvascularised cartilage-like template into such an environment should not result in cell death as would normally happen. Thirdly, endochondral ossification is characterised by attraction of vessels by the hypertrophic chondrocytes which occurs simultaneously with bone formation. This is the opposite of the intramembranous approach usually used in tissue engineering whereby bone formation is stimulated by an increase in vessel density which has already occurred. Combination of this approach with new scaffolds, could offer huge potential both for the fields of bone and osteochondral tissue engineering. The aim of this symposium is to discuss the recent advances in tissue engineering and regenerative medicine approaches related to bone formation and repair by investigating the role endochondral ossification has to play. It has

become apparent recently that endochondral ossification might lead to improved bone formation in vivo 1-9. This area of research is as a result becoming increasingly popular and promising. Several research groups are now focusing on endochondral ossification as a possible route to better bone repair in vivo as referenced above.

### **Engineering Angiogenesis**

Engineering functional capillaries, either in vitro or in vivo, is a major goal of regenerative medicine. This symposium will bring together biomaterials scientists and tissue engineers whose goals are to create matrices that are conducive to angiogenesis, or who develop in vitro models of angiogenesis and lymphangiogenesis for basic biology studies.

### **Engineering Cellular Microenvironments *in Vitro***

The microenvironment of cells is represented by other cells, and in particular in multicellular organisms, extracellular matrix. In a developing organism, or in cellular assemblies engineered in vitro, tissues emerge from coordinated sequences of cell renewal, differentiation, and assembly that are orchestrated by spatio-temporal gradients of regulatory factors. The biochemical composition, architecture, and the biomechanics of the cellular microenvironment act in concert to provide the necessary cues regulating cell function in the developing and adult organism. With recent major advances in stem cell biology, and a huge body of literature from the field of matrix biology tissue engineering is becoming increasingly oriented toward biologically inspired in vitro cellular microenvironments designed to guide stem cell growth, differentiation, and functional assembly. These microenvironments can be emulated by totally artificial means using scaffold systems and by creating surfaces that are modified and grafted with (bio)chemical cues and with defined mechanical properties. One aspect of these artificial systems are recent observations in lineage-directing effects of nanotopographical cues. The other extreme is not to control the composition and assembly of these microenvironments directly, but to control the culture environment of differentiated and stem cells which in turn will create their own extracellular matrix – and reliably so. This approach is based on the notion that cells are professional matrix makers and assemble larger aggregates together with ligands and stored growth factors with a precision and stoichiometric efficiency that is still unsurpassed. While the biomaterials field has been making large strides to synthesise, modify and to structure scaffolds serving as microenvironmental equivalents, advances in culture technology employing macromolecular crowding have allowed only recently to deposit matrix in vitro with an unprecedented efficiency. In the general context of tissue engineering, this symposium shall discuss the environments for guiding stem cell function and the different ways to derive them with a focus on the interplay between molecular and physical regulatory factors and the role of biochemical cues. The microenvironment theme is absolutely contemporary and will have a huge impact on the scientific studies of stem cell behaviour in the body or after ex vivo cultivation and re-administration (niche, homing). It is also crucial from the biotechnology platform point of view as growing stem cells ex vivo and differentiating them in an efficient way is absolutely dependent on their growth conditions and the microenvironment in vitro. Finally, tissue engineering of the cancer stem cell niche is becoming a hot topic together with the notion of the “carcinogenic matrix”. Tissue engineering forms the bridge here between studying the biology of stem cells and their application in regenerative medicine.

### **Engineering Complex Tissue Structures**

#### **Engineering Regenerative Environments *in vivo***

The development of paradigms, strategies and systems to define the cellular environment is critical for the successful implementation of regenerative therapies in the clinic. Replicating cellular microenvironments that promote healing response over scarring requires control over complex signal cascades, and precise phenotypic evolution of multicellular systems. Both in vitro and in vivo systems have an important role to play with respect to understanding the impact of biomaterial–exogenous signal juxtaposition in neo-tissue

development. Toward this end one can envision the development of engineered macromolecules (biomimetic polymers, peptides), materials that can remodel in response to changes in the biological environment (adaptive materials), smart delivery systems that recognize an evolving tissue environment and deliver precise sets of signals (synthetic signalling engines), and structurally defined nanomaterials, which by virtue of the physico-chemical and biological properties are capable of imposing strict outcomes in cell differentiation and tissue remodeling. The objective of this symposium is to highlight the recent developments in engineering cellular microenvironments, with an emphasis on systems that can impose control over cell differentiation both in vitro and in vivo. Specifically, the development of paradigms and systems that can be readily adapted to minimally interventional strategies will be highlighted. Additionally, the role of cellular-environment biomechanics in promoting tissue differentiation and functional recovery during healing will be covered. With this regard, the role of concomitant mechanical stimulation and exogenous signalling in the reparative process will be highlighted. Also, the role of material micro structure in tissue morphogenesis particularly with respect to controlling cell infiltration, angiogenesis and innervation will also be covered. The scope of the proposed symposium is very relevant to the translation of laboratory findings into the clinic, and it addresses the critical paradigm shift from engineering cell-based grafts to design of in situ regenerative strategies. It is anticipated that topics covered in this symposium will promote the discussion on how regenerative environments can be fostered and what are the key elements required to promote healing.

### **EuroSTEC: Soft Tissue Engineering for Congenital Anomalies**

EuroSTEC is an Integrated Project (IP) on ‘Soft tissue engineering for congenital birth defects in children: from ‘biomatrix - cell interaction - model system’ to clinical trials’, funded by the European Commission under the Sixth Framework Programme (FP6). The project brings together 15 partner organisations (10 research institutes and 5 companies) from 9 European countries. Modern tissue engineering approaches are used to develop new treatment options for children with structural disorders present at birth, such as spina bifida (skin), urogenital defects, gastroschisis, diaphragmatic hernia and esophageal atresia. A translational route through in vitro and animal experiments should finally lead to early clinical trials. Ethical and regulatory issues are addressed and a dialogue with society, including patient’s associations is sought. The project was inaugurated January 1st 2007 and involves 5 research areas with ten work packages and a separate work package on project management. In Research Area 1 collaborations were established between the different partners with exchange of knowledge and natural and synthetic biomatrix materials and the construction of new combinations evaluated. In Research Area 2 the evaluation of the current biomaterials in different culture systems was evaluated. In Research Area 3 new protocols were developed and new animal studies were performed for the evaluation of biomaterials in different animal models (rats, rabbits, pigs, sheep and fetal lambs). Also are new evaluation methods being explored. In Research Area 4 clinical trials are performed and evaluated on the selection criteria for patients for fetal interventions for CDH. Ethical approval was obtained for a new fetal intervention trial for CDH and the first patients are included. Prototypes of new instruments are developed and used in clinical procedures. In Research Area 5 a literature research on ethical issues was started and new insights discussed. A first evaluation of a Delphi round has taken place and focus group interviews have been performed. A database and data collecting system was established in order to be able to evaluate the options for European based studies and data collections. A start was made with a data collection system for animal studies. The project website ([www.eurostec.eu](http://www.eurostec.eu)) is showing updates, both with public information as well as information only accessible for the consortium members. Presentations by different partners will reflect the progress and developments in the different fields of expertise as well as the results of the interaction between the different partners and research groups.

### **Extracellular Matrix: Friend and Foe in Tissue Engineering**

Collagen and its many isoforms are the oldest extracellular matrix (ECM) components in the animal kingdom and has arrived with the evolution of multicellular organisms. Together with laminins, nectins, microfibrillar proteins, proteoglycans and associated ligands it forms a plethora of structures that lend typical form, structure and mechanical properties to different tissues. Different ECM molecules make up

connective tissue which functions as a glue giving cohesion of different layers of tissue and cell types, as insulation and filtering systems, but also as scaffold and niches. Besides its mechanical task, it also works as a notice board by storing and adsorbing signalling substances such as growth factors and thus can direct cellular functions. Without proper ECM development in vitro tissue engineered constructs would fall apart, a current problem in cell printing. Collagen does not only play a role in tissue development, but also in tissue repair. Collagen I in particular plays also a major role in wound healing. Conserved throughout evolution, the replacement of specialised tissue with collagen I – scarring – represents a quick fix. Scarring is a typical end stage of any form of wound healing or chronic inflammation in humans. The goal of this process is to regain tissue cohesion and to continue living. If the physiological scarring process goes awry fibrosis ensues, an excessive accumulation of collagen that leads to a broad spectrum of pathologies, ranging from cosmetic disfiguration to the failure of whole organs. Fibrosis around implants is an often encountered phenomenon – most spectacularly seen in breast implants, but this process affects virtually all implants. An acute wound is unavoidably created during the implantation process, but chronic inflammation can occur when the host organism interrogates the implant, and when gross differences between the mechanical properties of the implant and surrounding tissue exist. In any case, myofibroblasts are stimulated to move into the implant's vicinity and to lay down large amounts of collagen. This leads to an avascular fibrotic sheath or capsule around the implant. To give one example, current work on implantable glucose sensors is impeded by this tissue reaction. Besides the fast vascularisation of biomaterials and tissue –engineered constructs, fibrosis is one the major bottlenecks in tissue engineering and repair. Scarring hinges on collagen biosynthesis and its extracellular deposition and several strategies of how to deal with fibrosis shall be discussed and presented in this symposium.

### **Gene Therapy and Regenerative Medicine**

Gene therapy has immense promise for the treatment of a wide variety of diseases and disorders, but its realization requires major improvements in the actual mechanics of introducing exogenous DNA into somatic cells. Specifically, vectors are required which allow: reasonably efficient transfection; controllable expression period; cell or spatial specificity; and which have minimal side effects. Furthermore, these vectors should be mass-producible and relatively inexpensive if they are to have clinical impact. Viral vectors seem to satisfy at least the initial requirements, as most viruses can efficiently deliver nucleic acids and the transgene expression period is a function of the virus type. However, the success of viral vectors is limited due to their potential immunogenicity and oncogenicity, as well as their unsuitability for mass production. Despite these disadvantages, viral vectors are being used in numerous clinical trials including the diseased heart and orthopaedic applications. Nevertheless, despite relatively lower transfection efficiency, non-viral vectors have become increasingly more attractive. Interestingly the combined use of biomaterials and (viral and non-viral) vectors has become more and more interesting for the treatment of various diseases.

### **Imaging Technologies for Regenerative Medicine and Tissue Engineering Applications**

Bioengineering of a functional tissue or organ, by seeding living cells onto a biodegradable scaffold and then surgically implanting the construct into a patient, involves extensive remodeling of cells and scaffolds. A major barrier to progress has been the inability to monitor this dynamic complex biological process in real-time, which makes control and optimization extremely difficult. Without question then, development and implementation of technologies for nondestructive (i.e., noninvasive) imaging of regenerating tissues is an absolute prerequisite to the improved understanding and application of tissue engineering and regenerative medicine. In this regard, significant advances have already been made with a variety of imaging technologies, but the full potential of these technologies to tissue engineering and regenerative medicine have not yet been realized. The overall goal of this symposium is to describe and present new imaging technology innovations for tissue engineering and regenerative medicine applications. Several imaging technologies have already been applied for tissue engineering including: MRI, In vitro and in vivo fluorescent imaging, Optical Molecular Imaging, PET, X-ray, CT, Etc. The symposium will bring experts to review the latest developments in imaging technologies. We hope to bring together engineers and biologists to discuss the utilization of new imaging tools for tissue engineering applications. The overall

importance is related to the expectation that these critical enabling technologies will have a major and lasting impact on many areas/aspects of regenerative medicine and tissue engineering.

### **Injectable Scaffolds for Tissue Engineering**

Injectable scaffolds have attracted more and more attention in tissue engineering and regenerative medicine fields because of their promising advantages over performed scaffolds, which include ease of application, confined delivery for a site-specific action and improved patient compliance and comfort. In addition, injectable scaffolds mixing with cells form a uniform distributed cell/scaffold tissue system in vivo, which provide a rich and complex environment of signals so as to promote tissue regenerate and development. Several types of scaffold have been utilised as injectable scaffolds, which include both natural materials, such as: alginate gel, alginate tricalcium phosphate,  $\beta$ -tricalcium phosphate, calcium phosphate, chitosan, collagen, fibrin, fibronectin, gelatine; and synthetic or semi-synthetic materials, such as: poly(D-L-lactic acid) (PLA), poly(glycolide acid) (PGA), poly(D-L-lactide-co-glycolide acid) (PLGA), poly(propylene fumarate), poly(propylene fumarate)-diacrylate (PPF-DA), poly(caprolactone fumarate) (PCLF), poly(D-L-lactic-co-glycolic acid)/poly(ethylene glycol) (PLGA/PEG), and collagen/polymethylmethacrylate etc. However, many challenges in the design and development of injectable scaffold-cell system still remain:

- optimisation of the kinetics of solidification process in-situ;
- development of injectable scaffold that can withstand the mechanical stresses in the joint while maintaining an environment conducive to tissue growth;
- optimisation of creation of required pore size and formation of inter-connectivity of pores within the solidified scaffold;
- protection of cell damage and viability during preparation of scaffold and delivery;
- controlled release of growth factors;
- understanding of cell-material interaction, “cell-adhesion” and biocompatibility;
- rate of biodegradation and cytotoxicity;
- phenotype tissue formation.

As a consequence, a symposium based in this area would fit well with the key theme of the conference. The symposium is aimed at bringing together all aspects of the field, from synthetic chemists interested in developing suitable materials to applied scientists, engineers and clinicians that are interested in the applications of injective scaffolds. This symposium will cover following aspects of the state of art studies and developments of injectable scaffolds:

- Design and synthesis of suitable biomaterials for injectable scaffolds including hydrogel and stimuli materials;
- Functionalization and degradation mechanism studies of polymers for injectable scaffolds;
- Application and evaluations (in vitro and in vivo) of polymers for injectable scaffolds.

### **Mesenchymal Stromal Cells: Characterization and Expansion**

It is a strong belief that new modalities in tissue engineering and regenerative medicine will include cellular therapies involving the delivery of Mesenchymal Stromal Cells (MSCs). Currently there is no consensus among scientists regarding a method for the isolation of these cells from human tissue. There is some consensus on what are the defining characteristics of MSCs, based on a limited surface antigen profile, plastic adherence and multipotent differentiation. This is a somewhat slender list of attributes, none of which is specific to MSCs and the lack of agreed clinical release specifications is a serious impediment to progress in assessing the therapeutic potential of these cells in humans. There is a risk that current clinical studies will be rendered useless by the lack of consensus on the definition of MSC and the lack of standardised isolation protocols. This symposium will address a number of topics relating to current thinking on the characterization of the MSC. This will include an assessment of current experimental strategies directed towards understanding the bone marrow niche in which the MSC resides and the biological characteristics of the cell in vivo. Further aspects that will be discussed include methods that can be applied for the direct selection of cells from marrow aspirates, and the influence of culture conditions on the cell phenotype. Since MSC preparations are likely to consist of mixtures of progenitors and non-progenitors several attempts at isolating clonal populations have been described. This aspect will be included. Furthermore the effects of expansion on this heterogeneity will be discussed.

## **Model Systems of Development and Pathology**

### **Multifunctional Bone Scaffolds with Angiogenic and Therapeutic Potential**

The design, fabrication and characterisation of innovative scaffold systems for tissue engineering are attracting increasing interest in the fields of traditional and novel biomaterials. New concepts and related processing technologies are being focused on the development of multifunctional scaffolds (next generation scaffolds) which can have a drug delivery or biomolecular signalling function thus providing enhanced support to cell attachment, growth and proliferation beyond the classical function as mechanical support and temporary extracellular matrix. A range of polymeric, ceramic and composite matrices, usually combining natural and synthetic materials and biomolecules, are being developed which exhibit not only the required tailored 3D porous morphology but also controlled nanostructured surfaces. The symposium will offer an excellent forum to present and discuss the most recent and relevant contributions to the advancement of this attractive and innovative field, bringing together material scientists, biologists, pharmacists, tissue engineers and medical doctors. This symposium will be focused on the application of novel biomaterials and technologies for the development of advanced tissue engineering scaffolds for bone substitution and guided bone ingrowth, with angiogenic and therapeutic potential. The symposium will cover a wide range of topics, including the optimization of dedicated biomaterials, the development of new bone replacements following the principles of tissue engineering and surface functionalization exhibiting both osteogenic and angiogenic potential, the application of scaffolds as drug delivery platforms for therapeutic tissue engineering, the development of integrated scaffold processing technologies capable of producing multifunctional scaffolds and their application in experimental in-vitro and in-vivo models. In addition, papers considering the incorporation of extra functionalities in novel scaffolds, such as electronic or magnetic conduction, or self responsive function, will be also invited.

Suggested topics are (but not limited to): 1. Biomaterials promoting osteogenesis and angiogenesis, 2. Composite scaffolds with added functionalities, 3. Drug delivery scaffolds for therapeutic tissue engineering, 4. Osteoinductive and bioresorbable materials for bone replacement, 5. Optimization of material properties and their surfaces, including functionalization with biomolecules and drugs; 6. Advanced design and manufacturing techniques; 7. Contribution of nanotechnology to 3D scaffold development. 7. Scaffolds with therapeutic ion delivery.

### **Multi-Scale Hierarchical Scaffolds for Connective Tissues**

This symposium is focused in multi-scale hierarchical scaffolds developed for connective tissues. We aim at creating an excellent forum for the debate of the cutting edge research on multi-scale technologies applied to the development of new, smarter and super effective scaffolds able to overcome clinically-oriented unsolved problems in the area of connective tissues. The topics covered in the symposium will span from well established scaffold processing technologies to elegant new technologies maximizing the efficacy of co-cultures of cells combining controlled and sustainable release of drugs and bioactive molecules, with surfaces modified to generate specific biological functionality and mimicking the structural and biochemical features of the native extra-cellular matrix of connective tissues. This forum will unfold the unique properties of biomaterials across scales and the opportunities derived from the control of its structure and surfaces. Namely, the possibility to design scaffolds mimicking closely the motifs of the extra-cellular matrix or interacting with this milieu at the same length scale will enable tailoring the scaffolds to maximize its efficacy when in contact both with biological fluids and with relevant cell populations both in-vitro and in-vivo.

### **Nanotechnology for Regenerative Medicine**

Nanomedicine can be defined as the application of nanotechnology for health problems. It is known that the population of the Western world is ageing. During the next decennia an increase is expected in the population with an age in between 70-85 year. As a direct consequence, there will be an increase in

diseases that can be associated with ageing, like different types of cancer, diabetes, joint problems, tooth loss, etc. Those maladies do not have only a negative effect for the patient, but will also have a significant impact on our health care system. Therefore, it is extremely important that more active, less traumatic and less expensive methods and techniques are developed for the treatment and curing of these diseases. The expectation is that nanotechnology will provide an important contribution to the development of such techniques. Implants and tissue substitutes are made from (bio)materials that have one common property, i.e. biocompatibility. A promising application of nanotechnology is the development of better functioning biomaterials. The currently available biomaterials as used for the manufacturing of implants and tissue substitutes do not fulfill completely to its intended function. This is due to an un-natural response between the biomaterial and the surrounding tissue cells. A recent approach to the design of next-generation tissue regeneration supporting biomaterials is focusing on the more dimensionally intricate characteristics of surfaces, i.e. structure at the nanometer scale. The underlying hypothesis is that nanometer structure matches with the natural extracellular matrix resulting in an improved interaction of the tissue-forming cells compared with conventional biomaterials. Recent developments in the field of nanotechnology offer powerful tools to modify the surface of biomaterials by introducing artificial topography and specific surface chemistry on the material. It is well-known that both topography and surface chemical composition affect the reactions of the biological environment to the device. In this symposium, strategies and challenges for nano-assisted regenerative medicine are discussed.

### **Novel Membrane Concepts for Tissue Engineering Applications**

Membrane technology is of major importance in a number of life saving treatment methods. Membranes are used in drug delivery, diagnostic devices, as coatings for medical devices, bio-separations, artificial organs, tissue regeneration, etc. In the proposed symposium; novel membrane concepts for tissue engineering will be presented. We will have an interesting and well balanced program with presenters from academia and industry. In fact; we anticipate lectures on membranes for designing novel scaffolds; development of tissue engineering bioreactors; effects of membrane structure (micro- and nano-topography; porosity etc) on cell behavior. Besides; development of novel materials with improved biocompatibility / haemocompatibility for bio-artificial organs and scaffolds for tissue engineering will be discussed.

### **Polymer Materials Tailored for Tissue Engineering- European Society for Biomaterials Sponsored Symposium**

In the field of biomaterials research, polymer materials are a key contributor. It is often the case that players in the field use commercial polymers with ample knowledge of the material properties. It is therefore of importance that scientists active in the field of biomaterials, i.e. materials for regenerative medicine appreciate the basic properties of polymers and the concepts of polymers tailored for biomedical applications.

### **Preclinical Studies and Clinical Trials of Tissue Engineering**

Injuries and degenerative diseases constitute a bottleneck in both medical and surgical practice. Failure to restore damaged tissues leads to loss of function, impaired sensation and mobility and painful disorders. The healthcare costs are also enormous. It is therefore of paramount importance to develop strategies for functional tissue regeneration. Efforts to treat tissue injuries and degeneration have primarily focused on improving surgical techniques to provide a more robust repair and essentially a better quality of life. In many injuries, autografts, allografts and xenografts are considered to be the 'gold standards'. However, the limited supply of autografts and certain donor site morbidity restrict their utilisation. The use of allografts and xenografts has also been questioned due to increased possibilities of immune rejection and potential transmission of disease. A biomaterials-based tissue engineering approach was pioneered as the solely valuable alternative to transplantation crisis by fabricating constructs that will replace injured or degenerated tissues and restore function. In the quest of the ideal raw material for scaffold fabrication,

numerous synthetic and natural polymers have been investigated the recent years with favourable results. Advancements in cell and molecular biology indicate that the success of tissue engineering to restore function of injured or degenerated tissues depends on the choice of suitable cells to assure the persistence and function of the regenerated tissue. To this end, tissue engineering uses a variety of cells alone or seeded on a scaffold material as an essential part of the regeneration process. This symposium aims to outline what have we learned up to now from preclinical trials and how can we translate our findings to clinical applications. Our aim is to find out how far away are we from shelf-based therapies.

### **Regenerative Therapies for Osteoarthritis**

Human mesenchymal stem cells (hMSCs) represent a particular stem cell niche in the stromal compartment of the bone marrow, and consist of those stem cells that can differentiate into cells of mesenchymal tissues, including osteoblasts, adipocytes and chondrocytes. Osteoarthritis is the most common musculoskeletal disorder and extracts a significant social and psychological drain on those affected as well as those who care for them; in addition it incurs significant economic costs. This disease is characterised by articular cartilage degeneration and damage to the underlying subchondral bone. To date, there is a lack of effective therapies to treat the disease, resulting in total joint arthroplasty as the only viable therapeutic option. Thus, there is a need to develop methods that are less invasive and capable of regeneration of articular cartilage. The use of autologous chondrocytes in tissue engineering applications harbours a number of limitations in terms of efficacy and safety resulting in mesenchymal stem cells (MSCs) being considered an ideal therapeutic candidate for chondral repair. MSCs acting through trophic mechanisms are also known to prevent OA progression after intraarticular injection. The potential value of cellular therapies for tissue repair has been discussed and it is widely anticipated that new strategies will emerge in the near future. This symposium will discuss current trends, obstacles and gaps. This symposium will address recent advances in 1) animal models for OA, 2) how these models are used to increase our understanding of the disease and 3) how tissue engineering strategies, both traditional using cell-loaded scaffolds and the use of novel nanomaterials to target stem cells to areas of cartilage degeneration.

### **Replace, Repair or Regeneration Therapies in the Eye**

The eye has been the focus of significant advances in translational research both at the cellular and tissue engineering level. The symposium will reflect these advances with talks covering different diseases of the eye. Numerous corneal complications have been treated by corneal transplantation which is one of the most successful transplant procedures to date and is now also where successful adult stem cell transplantation procedures are being performed. The replacement of cataract lens with an artificial lens is a procedure which has recovered vision in millions of people worldwide but still remains one of the leading causes of blindness in the world. As such, the development of different artificial lenses remains at the forefront of Ophthalmology research. New cell based therapies are emerging using both embryonic and adult stem cells to repair or replace specific cells within the neural retina and its support tissue. These techniques are encompassing the latest cell biology and biomaterial advances and are at the forefront of translational research in the hope of treating numerous retinal diseases such as Age-related Macular Degeneration.

### **Smart and Self-assembling Systems in Tissue Engineering**

There is an increasing interest on the use of soft-based systems for various biomedical applications, including drug delivery, cell encapsulation and tissue repair. Among them, injectable fluids that can be introduced into the body in a minimally invasive manner prior to solidification or gellation within a specific site are very attractive in the context of tissue engineering and regenerative medicine. Different therapeutic agents, including growth factors, and cells can be incorporated by simple mixing. The solidification/gellation process occurs through chemical or physical changes, including self-assembly. Initial formulations may exhibit an intrinsically bioactive character, e.g. containing cell adhesion segments/elements, and their conception may be based on biomimetic strategies, e.g. using synthetic polypeptides or natural based macromolecules. The introduction of stimuli-responsive hydrogels has further strengthened the link between therapeutic need and release of bioactive agents or cell-material

interactions. Appropriate synthetic routes allow to design polymeric systems that can react within defined variations of single or multiple signals (temperature, pH, ionic strength...). Such systems can also be produced as thin films, coating the surface of biomaterials, in order to control cell adhesion upon changes in variable such as temperature or light or to control transport properties. Beside the reaction to external variables, hydrogels that can respond to cell stimuli have also be recognized as useful systems where, for example, the degradation of the material is controlled by the proliferation of the cellular content. Concepts currently used in Tissue Engineering (TE) applications are often based on traditional materials and processing methodologies. Such biomaterials are not able to establish specific interactions between the surface of the TE devices and the surrounding living tissues. In order to overcome this major drawback of TE should consider a new generation of biomaterials that will provide the adequate cues for cells, such as molecular and surface signals, to direct the correct phenotypic cell functions and spatial organisation. In nature, the biological world is built up via precise self-assembly of biomacromolecules. Indeed, examples of both static (phospholipid bilayers, protein folding) and dynamic (actin filaments, protein aggregated in signalling pathways) self-assembling processes are long time ago known to be related with cellular processes and structures. It has served as the main inspiration for the adoption of self-assembly methodologies in TE as a tool to try to reach the hierarchical and complex levels found in living tissues. This symposium will also address the use of molecular systems (both low molecular weight and macromolecules) that are able to form three-dimensional organizations or to modify the surface properties of substrates by means of self-assembling. For example, self-assembling peptide-based biomaterials have being developed for use as 3D tissue engineering scaffolds and for therapeutic drug-release applications.

### **Strategies for Intervertebral Disc Regeneration (EU Project)**

Causes of lower back pain are multifactorial, but in the majority of cases it is linked to clinical evidence of IVD degeneration. As a consequence, curing disc degeneration is one of the most important socioeconomic imperatives facing modern health care. 30% of European workers experience back pain, and it is the most frequently reported work-related disorder. The proposed workshop seeks to provide information on a material - cell- and clinical strategies to cure lower back pain, due damaged intervertebral disc (IVD), by enabling its regeneration to a natural healthy state or better. Cellular and acellular scaffolds designed by biomimetic approach needs to be designed to confer the appropriate mechanical and biological properties and enable the inclusion of the requisite cell signalling factors to produce a bio-hybrid structure which closely resembles the human tissue in all its essential attributes. Attention will be paid to the control of angiogenesis. In IVD tissue, vascularization must be carefully controlled, due to the unique anatomy and physiology of the intervertebral disc. There must be negligible vascularization in the annulus and nucleus regions and moderate vascularisation at the vertebral body level. Strategies on the functionalization of scaffolds, and on growth factor incorporation and delivery, are necessary to define different approach to enable, in this region,-specific control of vascularisation at different levels. Natural IVD tissue contains a relatively low number of cells, which are chondrocyte-like. Suitable and more readily available alternative cells and related techniques for incorporation in the bio-hybrid substitutes is an important issues to define appropriate regeneration kinetics.

### **Tissue Engineering Education: Ethics and Laboratory Teaching**

#### **Tissue Engineering Strategies for Rebuilding the Nervous System**

The symposium will feature presentations in the field of tissue engineering for nervous sytem regeneration. They have pioneered clinical applications for both naturally-derived and synthetic polymers that have been approved for use in patients. The focus of the presentations will be on the key scientific and regulatory questions that must be addressed in Europe and the United States for a new material and/or cell system to be introduced to human use.