PREFACE

The two broad categories of mammalian stem cells exist: embryonic stem cells, derived from blastocysts, and adult stem cells, which are found in adult tissues. In a developing embryo, stem cells are able to differentiate into all of the specialised embryonic tissues. In adult organisms, stem cells and progenitor cells act as a repair system for the body, replenishing specialised cells. As stem cells can be readily grown and transformed into specialised tissues such as muscles or nerves through cell culture, their use in medical therapies has been proposed. In particular, embryonic cell lines, autologous embryonic stem cells generated therapeutic cloning, and highly plastic adult stem cells from the umbilical cord blood or bone marrow are touted as promising candidates. Among the many applications of stem cell research are nervous system diseases, diabetes, heart disease, autoimmune diseases as well as Parkinson's disease, end-stage kidney disease, liver failure, cancer, spinal cord injury, multiple sclerosis, and Alzheimer's disease. Stem cells are self-renewing, unspecialized cells that can give rise to multiple types all of specialized cells of the body. Stem cell research also involves complex ethical and legal considerations since they involve adult, fetal tissue and embryonic sources. This new book presents the latest research from around the globe in this dynamic field.

Chapter 1 - Hematopoietic stem cells (HSC) are associated *ex vivo* with a cell subset actively rejecting lipocationic dye such as rhodamine123 (Rho) in the extracellular medium via the multi-drug resistance gene product. It is however not known whether this association remains after culture. The authors therefore evaluated whether cultures derived from CD34+ human cord blood (CB) cells contained dye-excluding (Rho-low) cells enriched in early HSC. Our investigations showed that after short-term culture with FLT3-ligand, thrombopoietin and stem cell factor, Rho-low cell were still detectable, but with a lower frequency than that of uncultured cells. Rho-low cell frequency varied depending on the stimulus used *in vitro*, and these variations correlated with that of long term-culture initiating cell (LTC-IC) frequencies (R=0.619, p<0.04), whereas no correlation could be established with late HSC (CFU) frequency. Rho-low cells sorted after 6 days of culture contained 2.8-fold more secondary CFU and 1.8 more LTC-IC compared to Rho-high cells. Though these data suggest that dye exclusion assays could be used as a relevant alternative compared to other *in vitro* assays to identify immature HSC after culture, it remains to be determined whether Rho-low cells derived from cultures retain clinically relevant characteristics such as the ability to reconstitute a lethally conditioned host. In this light, the authors further discuss below the

limitations of some *in vitro* and *in vivo* hematopoietic assays, and their relevance as tools to identify HSC that are able rescue conditioned patients.

Chapter 2 - *Background:* Clinical trials are ongoing involving transplantation of ex vivo expanded hematopoietic stem cells. This study was carried out in order to better understand the effects of ex vivo expansion on the rheology of umbilical cord blood-derived stem cells.

Methods: Micropipette aspiration and recovery experiments were performed using fresh umbilical cord blood CD34+ cells and umbilical cord blood CD34+ cells that were cultured for 1 week. From these experiments, viscosity and cortical tension were determined, and results were analyzed to determine if cultured cells differed from fresh cells in terms of rheology.

Results: As compared to fresh cells, cultured cells had a significantly lower viscosity (221.41±16.88 Pa•s as compared to 515.42±62.87 Pa•s for fresh cells, p=0.0001) and a significantly lower cortical tension (5.54±0.49x10-5 N/m as compared to 2.14±0.23x10-4 N/m for fresh cells, p<0.0001).

Conclusions: Cultured and fresh CD34+ cells are likely to behave differently in the body. Cultured cells are likely to flow more easily through the capillaries; however, they may also recover more slowly from a deformation.

Chapter 3 - Recent studies indicate that a variety of cells derived from adult human tissues show the capacity to change their tissue specific differentiation program. In this study the authors harvested cells from lipoaspirated adipose tissue, trabecular bone and pericranium tissue in order to evaluate and compare the differentiation potential of these populations by exposing them to adipogenic, chondrogenic, osteogenic and neurogenic induction media. Histological and immunochemistry analysis after adipogenic induction revealed adipocyte-like characteristic for all populations. Under osteogenic induction, all populations expressed the bone matrix proteins osteocalcin, osteopontin, osteonectin and secreted alkaline phosphatase, an early bone marker, into the culture medium. The chondrogenic potential was evaluated after staining of acidic glycoconjugates within the extracellular matrix. All induced cell populations were stained positive, indicating the presence of *de novo* cartilage. After neuronal induction, responsive cells exhibited neuron-like morphology with refractile cell bodies, extended long processes terminating in typical growth cones and long axon-like filopodia. Immunocytochemistry revealed the expression of neurofilament M and neuron specific enolase. Due to the use of heterogeneous cell populations each cell population preferentially expressed the phenotype of the tissue it was isolated from. In comparison; under the respective experimental conditions the periosteal and adipose derived cell populations demonstrated equal versatile developmental potentials according to cell morphology, immunocytochemistry and histology. In contrast the trabecular derived cells displayed a lower differentiation potential. These findings indicate that there are subpopulations within the isolated cell populations allowing all these cell preparations to express adipocyte-, osteoblast-, chondrocyte-, and neuron-like phenotypes.

Chapter 4 - Contrary to long-held dogma, neurogenesis occurs in the adult brain and neural stem cells (NSCs) reside in the adult central nervous system (CNS). This suggests that the CNS has the potential to self-repair. In support of this contention, neurogenesis is modulated in the adult brain, new neuronal cells are generated at sites of degeneration and injuries, after transplantation adult-derived neural progenitor and stem cells adopt the fate of the tissue, and adult stem cells may have the potential to differentiate into other lineages. Niches are microenvironments that control stem cell activity. The therapeutic potential of

stem cells, particularly adult NSCs, lies therefore in the interaction between stem cells and their niches. Neurogenic niches have been identified in the adult brain. Unraveling the interactions between NSCs and their niches will contribute to our understanding of the developmental potential of adult stem cells, and to bring adult NSC research to therapy.

Chapter 5 - With the passing of the Human Fertilization and Embryology (Research Purposes) Regulations 2001, the United Kingdom of Great Britain and Northern Ireland became the first country to pass legislation in support of embryonic stem cell research and research on embryos created by somatic cell nuclear transfer. While the UK legal stance and framework on embryo research are well-known and have attracted significant attention from those with an interest in the ethics of embryo research both in the UK and elsewhere, the arguments on the status of the embryo that were expressed by the members of Parliament and the main advisory bodies involved in this legal debate are less well-known. This article examines the entire range of arguments expressed in support of the Regulations. When the Human Fertilization and Embryology (Research Purposes) Regulations 2001 were passed, the UK had already established a legal framework on embryo research. Since this new legal stance must be understood against the background of these earlier developments and discussions on the status of the embryo, this article will also sketch the legal history of embryo research in the UK, documenting in particular how this legal history has been influenced by the Committee of Inquiry into Human Fertilization and Embryology's arguments on the status of the embryo. While the author argues that the validity of all the arguments can be questioned, the authors also argues that, as long as people have irreconcilable values, no case either for or against granting full moral status to the early embryo can be made that will convince everyone. At the same time, by clarifying my own position on the status of the early embryo, The author hopes to throw some light on why the author believes embryonic stem cell research should not be carried out. Therefore, this article will be of interest to all who have an interest in the ethics of embryonic stem cell research.

Chapter 6 - Inducible transgene expression remains an important challenge in embryonic stem cell research. It is desirable because it allows to control the level and timing of gene expression in stem cells. Here the authors describe a tetracycline-regulatable system based on the repression of a modified EF1-α promoter (EF1-αTetO2) by the native tetracycline repressor. EF1-αTetO2 activity was low in tetracycline repressor-expressing cell lines, but was strongly enhanced upon addition of tetracycline. Effective transgene expression was achieved within 24 hours of tetracycline addition; expression levels returned to baseline within 24 hours after tetracycline withdrawal. Graded transgene expression levels were achieved by increasing tetracycline concentrations. Efficient induction was observed in undifferentiated ES cells as well as ES cell-derived neurons. Upon subcutaneous cell implantation in mice, the system allowed in vivo induction of transgene expression. To our understanding, this is the first description of a switch-on expression system allowing transgene induction in implanted ES cells.

Chapter 7 - Mesenchymal stem cells (MSC) can be isolated from virtually all adult and fetal tissues and expanded in vitro. They share their origin in a perivascular niche suggesting a relationship with pericytes. Adult subcutaneous tissue is an abundant source of MSC, which share with bone marrow mesenchymal stem cell (BM-MSC) not only the capacity to differentiate into different mesenchymal lineages, but also an immunomodulatory effect. In view of the capacity to differentiate into different mesodermal tissues, the potential

application of adipose tissue-derived MSC (ATMS) in regenerative medicine as an alternative source to BM-MSC has raised great interest. In this chapter, the authors will discuss current data that suggest differences in phenotype, gene expression profile, osteo-chrondogenic potential, and hematopoietic supportive property between AT-MSC and BM-MSC.

Chapter 8 - Dendritic cells (DC) are a heterogenous population of bone marrow-derived cells residing in primary and secondary lymphoid organs. All DC share a common ability to process and present antigen to naïve T cells for the initiation of an immune response. However, they differ in surface markers, migratory patterns, localization and cytokine production. DC had been considered as myeloid cells for some time. However, recent findings have demonstrated that DC can develop not only from myeloid committed progenitors, but also from early lymphoid committed progenitors. The ability of DC to develop from progenitors committed to different lineages correlates with the surface expression of Flt3 receptor. The developmental events downstream from the lymphoid and myeloid committed progenitors as well as the molecular mechanisms required for DC development have been less well characterized. Recent studies have provided evidence for an intra-splenic precursor population that gives rise to the DC subsets *in situ*. The impaired DC development found in mice deficient in certain transcription factors known to be required for hemopoiesis has provided clues to the important role of transcription factors in regulating DC development. The recent finding that steady-state "dendropoiesis" can be recreated *in vitro* using bone marrow progenitors in the presence of Flt3 ligand will facilitate further studies of DC development and function. This review summarizes recent crucial findings on the development of a functional DC system.

Chapter 9 - There has been considerable progress towards the development of a stem cell-based therapy for Hirschsprung's disease (HSCR). This review article discusses the mechanisms regulating enteric nervous system (ENS) stem cell development and the molecular pathogenesis of HSCR in order to elucidate the feasibility of any stem cell therapy. Such a therapy will require a source of stem cells, techniques to isolate and amplify them, and effective methods for the transplantation of the stem cells or their progeny. To date, embryonic and postnatal ENS stem cells have been cultured as neurospheres prior to transplantion into mammalian bowel. However, while there are still many obstacles to overcome, recent observations have demonstrated functional changes in the gut after stem cell transplantation, indicating that a stem cell therapy for HSCR will indeed be possible.

Chapter 10 - The acute respiratory distress syndrome (ARDS) and acute lung injury (ALI) are common diagnoses in intensive care patients who require mechanical ventilation. They are acute in onset and typically fulminant in nature. No medical therapy has been found to improve survival from these disorders despite years of investigation and several clinical trials. Mortality for ARDS continues to approximate 40%. The alveolar epithelium, composed of types I and II cells, is a prime target for damage in ALI/ARDS, as is the pulmonary vasculature. Both the pulmonary epithelium and endothelium are injured diffusely. The development of pulmonary hypertension (PHTN) may be observed as well. Given the lack of available therapies for ALI/ARDS, investigators and clinicians alike are eager to explore new treatment modalities directed at repair of lung damaged in these disorders. As a result, the role of stem cells is receiving increasing attention. This review will focus on the role of stem cells in the repair of lung injury, specifically those studies that focus on the regeneration of lung epithelium and endothelium. Potential therapies for pulmonary hypertension will be

highlighted as well. Investigations that have been performed in animal models will be described, and how these may be applicable to human disease.