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STEM CELLS AND REGENERATIVE MEDICINE—THE EVOLVING STORY

Ann P. Chidgey, Natalie Seach, Rebecca Lim, Richard L. Boyd*

Monash Immunology and Stem Cell Laboratories (MISCL), Monash University, Melbourne, Victoria, Australia

INTRODUCTION

The continual quest of man to improve health care is not new—it is just the technologies that change. Over 2500 years ago, The Chinese developed substances to fight "infections" reaching through to quinine for malaria in the 17th century to 1877 when Pasteur and Koch found that an airborne bacillus had "antibiosis" actions, to Ehrlich's "antibiotic" for syphilis in 1909. But it was not until 1928 with Fleming's discovery of penicillin that the concept of antibiotics was popularised, ultimately culminating in a Nobel prize shared with Chain and Florey in 1939. The hallmark of defined antibiotics, coupled with major improvements in general sanitation, hygiene and nutrition, was the trigger to major improvements in human health and well-being. One of the ironies accompanying the increased lifespan of the population from approximately 40 years at the turn of 1900 to almost 80 years now is an unprecedented aging population and the onset of many types of degenerative diseases.

Globally, the aging society is not content with suffering, their impatience driving further expectations of better treatments and even cures. But this is counter balanced by the financially conservative nature of governments and their available input into health improvement. How do they triage the clinical needs before them? One of the issues is the time (10–20 years) and cost (hundreds of millions of dollars) involved to make the transition from "bench to bedside". Current estimates are that there are over 70 forms of intractable, degenerative conditions affecting hundreds of millions

* *ann.chidgey@med.monash.edu.au*

of people globally. It is this social and clinical burden that has been the catalyst to a new breakthrough technology, stem cell based therapies—a new revolution in medicine. Indeed this is best exemplified by the formation of the Californian Institute for Regenerative Medicine (http://www.cirm.ca.gov)—a $3 billion enterprise that is leading the world with the funding of stem cell research and its clinical applications.

The chapters in this book highlight the explosion in this area of research. For the first time, so many diseases have been covered under the same therapeutic umbrella—that provided by stem cells as the new foundation to regenerative medicine.

THE EVOLUTION OF REGENERATIVE MEDICINE

William Heseltine, Chairman and CEO of Human Genome Sciences (Rockville, MD), about 10 years ago described "regenerative medicine" as "*the broad range of disciplines . . . working towards a common goal of replacing or repairing damaged or diseased tissue.*" This section discusses the history and evolution of regenerative medicine and its associated paradigm shifts, as well as the interplay of factors that shaped the pace and direction of its development.

The earliest concept of tissue regeneration dates as far back as the ancient Greek mythologies. Hercules cut off the head of the monster Hydra and was shocked to observe two heads growing back in its place; Prometheus was chained to a rock where daily his liver was eaten by an eagle, but was renewed every night. In nature, newts are able to regenerate severed limbs and a number of lower vertebrates can regenerate central nervous system neurons following axotomy. Although humans are not capable of such dramatic regeneration, however on a daily basis the integrity of all cells, tissues and organs is homeostatically maintained by continual replacement from residual pockets of specific stem and progenitor cells. In many forms of tissue injury, adult stem cells both native to the organ as well as those distal to the site play a role in the repair and regeneration of the tissue. In the case of the skin, wound healing involves local cutaneous cells to reconstitute the epidermis as well as distant bone marrow derived cells, and the adjacent uninjured dermal mesenchyme to reconstitute the dermal fibroblast population [1]. In liver resection, up to two-thirds of the liver can be removed and remaining hepatocytes undergo replication to restore original organ size and function; however, under severe and sustained injury, intra-hepatic stem cells from the Canals of Hering become activated and take on the task of regeneration [2, 3].

While there have been suggestions of bone autografts for centuries and many sporadic attempts at transplantation surgery occurred up until the 1900s, the modern-day concept of transplantation as a paradigm for regenerative medicine did not become popularised until December 1967 with the first successful heart transplant performed by Christiaan Barnard. Interestingly, there were two main barriers to success—appropriate surgical procedures and immune rejection. The former is now stunning in its precision, while the latter still poses a major challenge.

Stem cell based therapies and their clinical applications to regenerative medicine have as their origins the world's first successful bone marrow transplant (BMT) in 1968, wherein whole bone marrow was transplanted into a patient and the stem cells contained within the graft successfully

replaced all haematopoietic cells [4]. While today's BMT benefits from extensive human leukocyte antigen (HLA)-typing, earlier unsuccessful attempts at BMT for the treatment of aplastic anaemia by Osgood in 1939 [5] preceded our understanding of the immunological factors involved in functional engraftment and graft-versus-host disease (GVHD). Early theories on the mechanisms of rejection were proposed by Ehrlich in 1906 and Carrel in 1910 [6, 7], who suggested that this rejection was due to malnutrition and physiological disturbances in donor tissues. As the ABO blood group system and immunological nature of allograft rejection in humans were discovered, more light was shed on the restrictions of transplantation, and hence the strategies required improving successful engraftment and long-term acceptance. However, despite the fact that it is now over 80 years since the first definition of major histocompatibility complex (MHC) antigens [8], the immunological barriers to successful, non-self transplants, including those of stem cells, remain a major challenge.

TISSUE ENGINEERING

Bioengineering is increasingly being recognised as an important component in the field of regenerative medicine. In the later part of the century, scientists faced yet another paradigm shift as tissue engineering was born from collaborative efforts by four independent laboratories at the Massachusetts Institute of Technology (MIT), which resulted in a number of products that are still available today [9]. Concurrent research at the Harvard Medical School to develop a bio-artificial pancreas [10, 11] led to a variety of biohybrid organ projects including Amcyte's and Novocell's Phase I/II trials of microencapsulated allogeneic islets cells. In 1993, *Science* published the first major review on tissue engineering [12] and by 1998, the US FDA approved the world's first allogeneic bioengineered product, Apligraf ®, which was marketed as a living skin equivalent.

As was the case for monoclonal antibodies and genetic engineering, initial claims for bioengineering were over-simplified and over-played. Suggestions that human organs could be grown in a petri dish resulted in unrealistic public expectations and media hype. The peak of this was perhaps in 2000 when *Time* magazine named tissue engineers as "The Hottest Job" for the future. There is, however, a re-incarnation of tissue engineering and its integration into creating artificial niches for stem cell growth.

NANOTECHNOLOGY

The concepts of nanotechnology were first introduced by Richard Feynman in 1959 [13] and further defined by Taniguchi in 1974 [14]. Today's interest in nanomedicine is based on the application of nanotechnology tools to the development of structures at the molecular level, which then allows for the improvement of interactions between synthetic materials and biological entities. Within the genre of regenerative medicine, nanoparticle research has mainly addressed the development of entrapment and delivery systems for genetic material, therapeutic agents, biomolecules and as a reinforcing- or bioactivity-enhancement phase for polymeric matrices in 3D

scaffolds. The advances in nanotechnology facilitate the synthesis of an extracellular matrix (ECM) that can promote and influence cell growth, cell mobility and adhesion—much more than mimicking a cell's natural environment. Micro- and nano-topography have been shown to be crucial cues for cell behaviour including differentiation [15, 16]. In the case of tissues such as tendons, nerves, corneal stroma and intervertebral disc regeneration, cell orientation using contact guidance afforded by nanoscaffolds is essential to achieve functional tissue [17, 18]. The capability of nanomedicine to produce nanostructures, which can mimic natural tissues, as well as nanoparticles in delivery systems has elicited research interest in this field. One of the challenges scientists are facing today is developing artificial nanocarriers that can pass tight junctions, blood–brain barriers and capillaries but with the efficiency and specificity similar to that of viruses [19].

STEM CELLS IN REGENERATIVE MEDICINE

Embryonic Stem Cell Therapy

Recently, human embryonic stem cells (hESCs) have been touted as heralding the dawn of a revolutionary age of regenerative medicine [20, 21]. Following the "discovery" of hESCs in 1998 [22], and their capacity for diverse differentiation [23], our understanding of the biology of these cells, their physiological requirements and the parameters of their potential has increased enormously. Protocols have been developed to maintain hESCs in their undifferentiated state, and panels of primitive markers related to "*stemness*" have been described to identify, sort and isolate the cells. The main characteristic that separates hESCs from all other types of cells is that they are truly pluripotent, that is, they can potentially give rise to virtually all cell types in the human body and, therefore, can provide exciting new therapies for tissue regeneration. Unlike haematopoietic and other adult stem cells that cannot undergo long-term self-renewal *in vitro* [24], hESCs appear to be capable of infinite self-renewal and can, therefore, expand to very large numbers [25].

Some of society's most devastating diseases, such as diabetes, kidney failure, lung disorders, spinal injury, stroke, neurodegenerative disorders, haematological disorders and heart failure, for example, may potentially be treated with defined cell populations able to repair or replace the damaged tissue. Whilst generating specific cell types from ESCs *in vitro* poses many challenges, the activation or inhibition of specific factors required to induce the differentiation of ESCs through the many developmental stages towards lineage-specific progenitors and mature cells, are slowly being unravelled [26]. Despite the enormous potential of ESCs, and the fact that many ethical issues have been overcome, their clinical utility has been tempered by safety concerns. These have arisen directly from their characteristic propensity for teratoma formation (tumours derived from all three germ layers) and whether therapeutic ES products do indeed faithfully recapitulate the normal cells and tissues they are designed to repair or replace. This does not preclude the value of ESCs for generating invaluable cell lines as diagnostics for drug testing. The issue of immune rejection will be a challenge with traditional ESCs.

Tolerance to Stem Cell Therapeutics

The immunogenicity of ESC transplants has been the topic of much debate. The discovery that ESCs expressed only low levels of MHC proteins—few MHC Class I and virtually no MHC Class II [27]—led to the possibility that ESCs might be "immune privileged", that is, they can elude the host immune system. However, compelling evidence of immune rejection has conclusively shown recently that this is not the case. Using a sophisticated non-invasive molecular imaging technique, Swijnenburg and colleagues [28] unequivocally demonstrated immune rejection of hESC within 10 days of transplantation into immune competent mice. Furthermore, upon re-injection of hESC, the already primed immune system rejected them within 2–4 days. This study suggests that patients will reject allogeneic hESC transplants, as they would for any other allogeneic solid organ transplant.

It was initially perceived that the environment in which ESCs would be transplanted into may induce the differentiation of these cells into the surrounding tissue type, with the caveat that aged niches may have reduced capacity to stimulate stem cell regeneration [29]. In this respect, Nussbaum and colleagues [30] confirmed that adult tissues may lack the cues to induce ESCs to form mature cell types. In carefully controlled experiments, syngeneic undifferentiated mouse ESCs were transplanted into normal and infarcted adult hearts. The damaged cardiac tissue lacked the required inductive cues for ESC differentiation into cardiomyocytes and teratomas formed. Furthermore, while the grafts were accepted in immunocompromised and syngeneic hosts, they were eventually rejected in allogeneic hosts [30].

Inducing ESCs to differentiate *in vitro* into the appropriate cell types prior to transplantation would avoid the issue of teratoma formation, provided there were no lingering undifferentiated cells. However, it is highly probable that the differentiated progeny of ESCs will have increased expression of MHC proteins (referred to as HLA), even more so in inflammatory environments, which stimulate the production of cytokines such as IFNg. This would increase their likelihood of immune rejection in an allogeneic setting. Clearly efficient tolerance strategies will need to be addressed to avoid life-long administration of immunosuppressive treatments, which are associated with high morbidity and loss of quality of life, with rejection ultimately inevitable.

Several alternatives to generalised immune suppression have been proposed in order to overcome immune rejection [26, 31, 32]. These include the accessibility of enough ESC lines to provide histocompatibility matching to the genetically diverse population, to engineered ESCs that suppress HLA expression or secrete immunosuppressive molecules. Each has its associated difficulties and risks. It was estimated that 150 different cell lines will be required to provide a full match at HLA-A, -B and -DR types for 20% of recipients, a beneficial match for 38% with one HLA-A or one HLA-B mismatch only, and one HLA-DR match for 84% [33]; immunosuppressive treatment will, therefore, be required even after that.

Another approach—using the same rules that establish and maintain self-tolerance, and has many examples of proof of concept—is to re-educate the immune system to accept donor tissue by creating haematopoietic chimeras. Patients receiving a bone marrow, or haematopoietic stem cell (HSC), transplant prior to solid organ transplants from the same donor were taken off

immunosuppressive treatments once donor engraftment was achieved (for example, [34–39]). Whilst successes in this approach have been described for over a decade, it is surprising that it has not been incorporated more often in clinical transplantation therapy, at least in young recipients who have a more robust immune regenerative potential. Thus, by deriving HSCs from the same hESC line that was used to develop the cell therapeutic, central tolerance could be established, provided the hESC-derived HSCs readily engraft into the bone marrow and then migrate as appropriate progenitors to the thymus. Here they convert to not only T-cells but also dendritic cells, which can deliver tolerogenic signals to the developing thymocytes, eliminating or silencing any that may be donor (or self-) reactive.

One limiting factor to such tolerance-inducing approach is the age of the recipient. This is of paramount importance as the vast majority of people requiring regenerative medicine-based therapies will be adults through to the elderly. However, the thymus—the function of which is critical for central tolerance—atrophies with age such that by mid-life it has less than 10% of its maximal functional tissue [40]. The bone marrow niche may also be altered with age, although this phenomenon is not as clear-cut as that evident in the thymus, with the possibility of both intrinsic and extrinsic effects altering HSC and B-cell developmental potential with age [41–47]. The realisation that damage from pre-conditioning regimes required for HSC transplants may further compromise the function of the thymus and bone marrow in the elderly has led to increased attention towards developing strategies to enhance T-cell reconstitution. Such strategies include regenerating the bone marrow [48] and thymic niches [49, 50], adoptive transfer of T-cell precursors [51] and induction of regulatory T-cells (Treg) [52–54]. Changes in the immune-endocrine axis with age have provided some insight into the mechanisms of thymic involution and these are currently being investigated as potential therapeutics for thymic regeneration. Blocking the suppressive effects of sex steroids by administering a luteinising hormone releasing hormone (LHRH) agonist has had dramatic results in regenerating lymphopoiesis and immune reconstitution in aged animal models and following chemotherapy-induced damage [55–60] and is currently showing positive results in clinical trials [61]. Growth factors, such as growth hormone, keratinocyte growth factor, interleukin-7 and Fms-like tyrosine kinase 3, have also shown potential as regenerative agents [62].

Strategies to produce patient-specific "embryonic" stem cells are being developed to avoid rejection, such as induced pluripotential stem (iPS) cell lines, generated by the dedifferentiation or reprogramming of patient somatic cells [63–66]. The reprogramming requires integration of retroviruses encoding specific transcription factors. The risks in using viral vectors are presently too great for clinical application; however, developing technologies for transient gene transfer or the transfection of proteins rather than genes may overcome this and the possibility of immune rejection, if viral antigens are present.

ADULT STEM CELLS

Although BMT has now been a very successful therapy for many decades, recently, in part instigated by the ethical and safety issues with ESCs, adult stem cells have become a major focus of

interest. It is now clear that every tissue and organ in this body has its own reservoir of stem cells that provide the "engine room" for homeostatic maintenance of the body. The aging process no doubt reflects a numerical or functional degradation in these stem cells—hence major efforts being made to identify and target adult stem cells for regenerative medicine.

Indeed pre-clinical studies are already being translated into the clinic. In particular, bone marrow derived stem/progenitor cells may also migrate to distant extramedullary peripheral sites after severe tissue damage and participate in repair, remodelling and the regeneration process [67–70]. This unique feature makes them a relevant source of immature cells for tissue repair based on the body's own regenerative capabilities [71]. The tissue regeneration mediated via adult stem/progenitor cells is usually accompanied by changes in the local environment orchestrated by growth factor and cytokine-initiated cascades including EGF-EGFR, Wnt/β-catenin, Notch, BMP, SDF-1-CXCR4 signalling pathways [72–74]. Additionally, adult stem cells are being studied as a novel means to deliver gene therapy, for example anti-angiogenic or cytotoxic agents can be directed to specific tumoural sites as a treatment for aggressive and metastatic tumours, which are unamenable to traditional therapy [75–78].

Two adult stem cells, namely the mesenchymal stem cell (MSC) and the amnion epithelial stem cell, have emerged recently in the literature as cells with marked potential in the realm of immunomodulatory and regenerative medicine. MSCs (also referred to as mesenchymal stromal cells) were originally isolated from the bone marrow but are now found in numerous tissues, including adipose, skin, umbilical cord and placental membranes. Isolated from their milieu on the basis of adherence to plastic and negative expression of haematopoietic markers, MSCs demonstrate clear multipotency for differentiation into cells of mesenchymal lineage such as adipocytes, chondrocytes and osteoclasts and are currently being used clinically for the treatment of genetic bone disorders such as *Osteogenesis imperfecta*, as well as mechanical bone and cartilage injuries (refer to Table 1.1). More controversially, MSCs have also reported trans-differentiation into cells of both endodermal [79, 80] and ectodermal germ layers [79, 81], indicating potential for the treatment of neurological conditions such as stroke as well as therapeutic applications in both pancreatic, renal and liver function [82]. An *in vivo* study has recently reported improved neurological function in a cohort of patients injected with autologous MSC for the treatment of multiple system atrophy [83]. In addition, multiple clinical trials are underway to assess the effect of site-specific injection of MSC in patients with end-stage liver disease as well as a broad range of cardiac myopathies (refer Table 1.1).

MSCs also function as potent regulators of the immune system, suppressing immune responses both *in vitro* and *in vivo* [84]. This remarkable property has, most notably, led to its clinical use in patients undergoing myeloablation for HSC transplant. Numerous studies have now demonstrated the powerful effect of MSC infusion in reducing the incidence and/or severity of GVHD in patients receiving allogeneic HSC transplantation, as well as improvement in donor stem cell engraftment and function [85–92]. The immunosuppressive properties of MSC are currently being evaluated as potential therapy for other immune disorders including Crohn's disease, systemic lupus erythematosus and Type 1 diabetes (refer to Table 1.1).

Amnion epithelial cells (AECs) are isolated from the amnionic membrane of discarded placental tissues. Placental amnion has been widely used in medical history for its pro-epithelial and anti-inflammatory properties, in particular for the treatment of both chemical and thermal burns and corneal defects [93]. In more recent times, AECs have been isolated as single cells from the epithelial layer of the amniotic membrane and have been shown to retain pluripotent properties similar to ESCs. Unlike ESCs, however, human AECs do not form teratomas when injected into SCID mice and thus represent a safer therapeutic option for the treatment of a wide range of tissue-related disorders [94, 95]. Ongoing research indicates that AECs retain the ability to differentiate into tissue cell types from all three germ layers. Currently, most of the work pertaining to AEC has occurred in pre-clinical animal models and demonstrates promising outcomes in a wide range of disorders including liver disease [95–97] and neural disorders [98–101]. A clinical trial is now underway to assess the effect of transplanted, culture-derived AEC in repairing damaged ocular surfaces (Clinicaltrials.gov identifier NCT00344708). Recent studies have also indicated immunosuppressive properties of AEC, similar to that observed for MSC [102]. Importantly, both MSC and AEC appear to be immune privileged and thus are not encumbered by the problems of immune recognition, allowing clinical MSC transplants of both autologous and "off-the-shelf" allogeneic therapeutic products. The ease of isolation and harvest of these cells types, such as bone marrow or adipose biopsies for MSC isolation and discarded, full-term placental tissue for AEC extraction, are both relatively non-invasive and herald minor ethical considerations. Both AEC and MSC represent a viable and promising source of cell therapy for regenerative medicine.

Table 1.1 ***Clinical Use of Mesenchymal Stem Cells—Published and Ongoing Clinical Trials***

Disease/condition	*Intervention*	*Patient number/ clinical phase*	*Reference/clinical trial identifier (Clinicaltrials.gov)*
Graft versus host disease	MSC infusion (I.V.), HLA-identical	1 patient	Le Blanc et al., 2004 [89]
	MSC infusion (I.V.), HLA-identical, haploidentical, HLA-mismatched	8 patients	Ringden et al., 2006 [92]
	MSC infusion, HLA-mismatched	2 children	Fang et al., 2007 [103]
	MSC infusion (I.V.), HLA-identical, haploidentical, HLA-mismatched	Phase II	Le Blanc et al., 2008 [88]
	MSC infusion, HLA-mismatched	1 child	Ball et al., 2008 [104]
	MSC infusion (I.V.), allogeneic	Phase I	NCT00361049
	MSC infusion (I.V.), allogeneic	Phase I/II	NCT00314483
	MSC infusion (I.V.), allogeneic	Phase I/II	NCT00447460
	MSC infusion (I.V.), Umbilical cord-derived	Phase I/II	NCT00749164
	MSC infusion (I.V.), OTI-010	Phase II	NCT00081055

(Contd.)

Table 1.1 (Contd.)

	MSC infusion (I.V.), Prochymal™	Phase II	NCT00504803
	MSC infusion (I.V.), Prochymal™	Phase II	NCT00603330
	MSC infusion (I.V.), Prochymal™	Phase II	NCT00476762
	MSC infusion (I.V.), Prochymal™	Phase II	NCT00136903
	MSC infusion (I.V.), Prochymal™	Phase II	NCT00284986
	MSC infusion (I.V.), Prochymal™	Phase III	NCT00366145
Haemopoietic engraftment			
Breast cancer	MSC infusion (I.V.), autologous	28 patients	Koc et al., 2000 [86]
Acute myelogenous leukaemia	MSC infusion (I.V.), HLA-mismatched	1 patient	Lee et al., 2002 [91]
Hematologic malignancy	MSC infusion (I.V.), HLA-identical	phase I	Lazarus et al., 2005 [87]
Leukaemia, aplastic anaemia, severe combined immunodeficiency (SCID)	HLA identical, haploidentical	7 patients	Le Blanc, 2007 [90]
Cardiac myopathy (total 7)			
Myocardial infarction	MSC infusion, Provacel™	Phase I	NCT00114452
Congestive heart failure	Intramyocardial MSC injection, autologous	Phase I/II	NCT00644410
Myocardial infarction	Intramyocardial MSC injection, autologous	Phase I/II	NCT00587990
Chronic myocardial ischemia	Intramyocardial MSC injection, autologous	Phase I/II	NCT00260338
Acute myocardial infarction	Transendocardial injection, allogeneic	Phase I/II	NCT00555828
Heart failure	Transendocardial injection, allogeneic	Phase II	NCT00721045
Heart failure	Intramyocardial MSC injection, autologous	Phase II	NCT00418418
Osteogenesis Imperfecta	MSC infusion (I.V.), allogeneic	6 children	Horwitz et al., 2002 [105]
	In-uterine transplantation of allogeneic MSC	1 patient	Le Blanc et al., 2005 [106]
	Bone marrow cell infusion (CD3 depleted), allogeneic	Pilot	NCT00187018
Chrohn's disease	MSC infusion (I.V.), Prochymal™	Phase II	NCT00294112
	MSC infusion (I.V.), Prochymal™	Phase III	NCT00543374
	MSC infusion (I.V.), Prochymal™	Phase III	NCT00482092
	MSC infusion (I.V.), Prochymal™	Phase III	NCT00609232

(Contd.)

Table 1.1 (Contd.)

Diabetes (type1)	-Cotransplantation of islet (allograft) and MSC infusion-autologous	Phase I/II	NCT00646724
	-MSC infusion (I.V.) in recently diagnosed type I diabetics, Prochymal™	Phase II	NCT00690066
Chronic obstructive pulmonary disease	MSC infusion (I.V.), Prochymal™	Phase II	NCT00683722
Multiple sclerosis	MSC infusion, autologous	Phase I/II	NCT00395200
End stage liver disease	MSC (differentiated into progenitor hepatocytes) infusion (portal vein), autologous	Phase I/II	NCT00420134
Systemic Lupis	MSC infusion (I.V.), autologous	Phase I/II	NCT00659217
Erythematosus	MSC infusion (I.V.), allogeneic	Phase I/II	NCT00698191
Decompensated cirrhosis	MSC infusion (I.V.), autologous	Phase II	NCT00476060
Tibial fracture	Local MSC implantation, autologous	Phase I/II	NCT00250302
Partial medial meniscectomy	Intra-articular MSC injection, autologous	Phase I/II	NCT00702741
Adult periodontitis	Implant of scaffold including MSC and ostoclasts	Phase I/II	NCT00221130
Multiple system atrophy	MSC infusion (intra-arterial and I.V.), autologous	18 patients	Lee et al., 2007 [83]

Note: I.V., intravenous.

CONCLUDING

From the earliest applications of regenerative medicine in BMT nearly five decades ago, this dynamic area of research has grown to include both adult and embryonic stem cells, fusing the continually evolving molecular biology with cutting edge sciences such as nanotechnology and tissue engineering. Thus regenerative medicine in this day and age incorporates both the transplantation of cells or synthesised material into the body, as well as aiding the body's natural regenerative capacity. It is not surprising that this field of science has expanded exponentially with our evolving understanding of signalling pathways vital to the differentiation of stem cells and better grasp of nanotechnology in order to build more complex scaffolds to include growth factors, ECM and other proteins. The prospect of *ex vivo* cell, tissue and organ genesis is conceivable.

Our ability to create better animal models for studying regeneration and graft acceptance has also played a vital role in the evolution of regenerative medicine. Tail amputations performed on the zebrafish model shed light on the role of Wnt/β-catenin in limb regeneration, and the production of "humanised" mice allows the testing of potential therapeutics in a system that mimics the human haematopoietic-lymphoid system, and although this does not exclude the need for large animal studies, they are vital in the speeding up of preclinical evaluation of novel agents.

While the potential for hESCs and adult stem cells in regenerative medicine is obvious, it is important to keep in mind the challenges that need to be overcome:

(i) allogenic transplantation of adult stem cells, hESCs and hESC-derived progenitors will still require donor tolerance induction by its recipient;

(ii) hESCs can form teratomas, for which grafts must be free of all undifferentiated cells prior to transplantation; and

(iii) the microenvironment of the grafted tissue must be conducive to the survival of the graft because the graft will be exposed to all the host factors that cause tissue damage in the first place.

Indeed the new revolution in regenerative medicine that the age of stem cells has instigated is tantalisingly close. It will be fascinating to revisit the contents of this excellent book in 5 and 10 years time—how accurate it was and what amazing discoveries are still in store!

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REFERENCES

1. Fathke C., L. Wilson, J. Hutter, V. Kapoor, A. Smith, A. Hocking, F. Isik (2004). Contribution of bone marrow-derived cells to skin: collagen deposition and wound repair. *Stem Cells*. **22**: 812–822.
2. Chen Y.K., X.X. Zhao, J.G. Li, S. Lang, Y.M. Wang (2006). Ductular proliferation in liver tissues with severe chronic hepatitis B: an immunohistochemical study. *World J. Gastroenterol.* **12**: 1443–1446.
3. Koenig S., I. Probst, H. Becker, P. Krause (2006). Zonal hierarchy of differentiation markers and nestin expression during oval cell mediated rat liver regeneration. *Histochem Cell Biol.* **126**: 723–734.
4. McCann S.R. (2003). In: *The History of Bone Marrow Transplantation*. N.S. Hakim, V.E. Papalois (Eds.) Organ & Cell Transplantation. Imperial College Press, London, UK.
5. Osgood E.E., M.C. Riddle, T.J. Matthew (1939). Aplastic anemia treated with daily transfusions and intravenous marrow; case report. *Ann. Intern. Med.* **13**: 357–367.
6. Baar H.S. (1963). From Ehrlich-Pirquet to Medawar and Burnet; A revolution in immunology. *J. Main. Med. Assoc.* **54**: 209–214.
7. Carrel A. (1910). The replantation of the kidney and the spleen. *J Exp Med* **12**(2): 146–150.
8. Gorer P.A. (1937). The genetic and antigenic basis of tumour transplantation. *J. Pathol. Bacteriol.* **44**: 691–697.
9. Bell E., B. Ivarsson, C. Merrill (1979). Production of a tissue-like structure by contraction of collagen lattices by human fibroblasts of different proliferative potential *in vitro*. *Proc. Natl. Acad. Sci. USA*. **76**: 1274–1278.

10. Chick W.L., A.A. Like, V. Lauris, P.M. Galletti, P.D. Richardson, G. Panol, T.W. Mix, C.K. Colton (1975). A hybrid artificial pancreas. *Trans. Am. Soc. Artif. Intern. Organs.* **21**: 8–15.
11. Chick W.L., J.J. Perna, V. Lauris, D. Low, P.M. Galletti, G. Panol, A.D. Whittemore, A.A. Like, C.K. Colton, M.J. Lysaght (1977). Artificial pancreas using living beta cells: effects on glucose homeostasis in diabetic rats. *Science.* **197**: 780–782.
12. Langer R., J.P. Vacanti (1993). Tissue engineering. *Science.* **260**: 920–926.
13. Feynman R. (1959). There's Plenty of Room at the Bottom. *American Physical Society Caltech*, CA.
14. Taniguchi N., "On the Basic Concept of 'Nano-Technology'," *Proc. Intl. Conf. Prod. Eng. Tokyo, Part II, Japan Society of Precision Engineering*, 1974.
15. Boyan B.D., L.F. Bonewald, E.P. Paschalis, C.H. Lohmann, J. Rosser, D.L. Cochran, D.D. Dean, Z. Schwartz, A.L. Boskey (2002). Osteoblast-mediated mineral deposition in culture is dependent on surface microtopography. *Calcif Tissue Int.* **71**: 519–529.
16. Zinger O., G. Zhao, Z. Schwartz, J. Simpson, M. Wieland, D. Landolt, B. Boyan (2005). Differential regulation of osteoblasts by substrate microstructural features. *Biomaterials.* **26**: 1837–1847.
17. Gomez N., Y. Lu, S. Chen, C.E. Schmidt (2007). Immobilized nerve growth factor and microtopography have distinct effects on polarisation versus axon elongation in hippocampal cells in culture. *Biomaterials.* **28**: 271–284.
18. Teixeira A.I., G.A. McKie, J.D. Foley, P.J. Bertics, P.F. Nealey, C.J. Murphy (2006). The effect of environmental factors on the response of human corneal epithelial cells to nanoscale substrate topography. *Biomaterials.* **27**: 3945–3954.
19. Mastrobattista E., M.A. van der Aa, W.E. Hennink, D.J. Crommelin (2006). Artificial viruses: A nanotechnological approach to gene delivery. *Nat Rev Drug Discov.* **5**: 115–121.
20. Braude P., S.L. Minger, R.M. Warwick (2005). *Stem cell therapy: Hope or hype? BMJ* **330**: 1159–1160.
21. Kitzinger J., C. Williams (2005). Forecasting science futures: Legitimising hope and calming fears in the embryo stem cell debate. *Soc Sci Med.* **61**: 731–740.
22. Thomson J.A., J. Itskovitz-Eldor, S.S. Shapiro, M.A. Waknitz, J.J. Swiergiel, V.S. Marshall, J.M. Jones (1998). Embryonic stem cell lines derived from human blastocysts. *Science.* **282**: 1145–1147.
23. Reubinoff B.E., M.F. Pera, C.Y. Fong, A. Trounson, A. Bongso (2000). Embryonic stem cell lines from human blastocysts: Somatic differentiation *in vitro*. *Nat. Biotechnol.* **18**: 399–404.
24. Wang L., P. Menendez, C. Cerdan, M. Bhatia (2005). Hematopoietic development from human embryonic stem cell lines. *Exp Hematol.* **33**: 987–996.
25. Avery S., K. Inniss, H. Moore (2006). The regulation of self-renewal in human embryonic stem cells. *Stem Cells Dev.* **15**: 729–740.
26. Murry C.E., G. Keller (2008). Differentiation of embryonic stem cells to clinically relevant populations: lessons from embryonic development. *Cell.* **132**: 661–680.
27. Drukker M., G. Katz, A. Urbach, M. Schuldiner, G. Markel, J. Itskovitz-Eldor, B. Reubinoff, O. Mandelboim, N. Benvenisty (2002). Characterisation of the expression of MHC proteins in human embryonic stem cells. *Proc. Natl. Acad. Sci. USA.* **99**: 9864–9869.
28. Swijnenburg R.J., S. Schrepfer, J.A. Govaert, F. Cao, K. Ransohoff, A.Y. Sheikh, M. Haddad, A.J. Connolly, M.M. Davis, R.C. Robbins, J.C. Wu (2008). Immunosuppressive therapy mitigates immunological rejection of human embryonic stem cell xenografts. *Proc. Natl. Acad. Sci. USA.* **105**: 12991–12996.

29. Carlson M.E., I.M. Conboy (2007). Loss of stem cell regenerative capacity within aged niches. *Aging. Cell.* **6**: 371–382.
30. Nussbaum J., E. Minami, M.A. Laflamme, J.A. Virag, C.B. Ware, A. Masino, V. Muskheli, L. Pabon, H. Reinecke, C.E. Murry (2007). Transplantation of undifferentiated murine embryonic stem cells in the heart: Teratoma formation and immune response. *Faseb. J* **21**: 1345–1357.
31. Cabrera C.M., F. Cobo, A. Nieto, A. Concha (2006). Strategies for preventing immunologic rejection of transplanted human embryonic stem cells. *Cytotherapy.* **8**: 517–518.
32. Drukker M., N. Benvenisty (2004). The immunogenicity of human embryonic stem-derived cells. *Trends Biotechnol.* **22**: 136–141.
33. Taylor C.J., E.M. Bolton, S. Pocock, L.D. Sharples, R.A. Pedersen, J.A. Bradley (2005). Banking on human embryonic stem cells: estimating the number of donor cell lines needed for HLA matching. *Lancet.* **366**: 2019–2025.
34. Alexander S.I., N. Smith, M. Hu, D. Verran, A. Shun, S. Dornery, A. Smith, B. Webster, P.J. Shaw, A. Lammi, M.O. Stormon (2008). Chimerism and tolerance in a recipient of a deceased-donor liver transplant. *N. Engl. J. Med.* **358**: 369–374.
35. Helg C., B. Chapuis, J.F. Bolle, P. Morel, D. Salomon, E. Roux, V. Antonioli, M. Jeannet, M. Leski (1994). Renal transplantation without immunosuppression in a host with tolerance induced by allogeneic bone marrow transplantation. *Transplantation.* **58**: 1420–1422.
36. Kadry Z., B. Mullhaupt, E.L. Renner, P. Bauerfeind, U. Schanz, B.C. Pestalozzi, G. Studer, R. Zinkernagel, P.A. Clavien (2003). Living donor liver transplantation and tolerance: A potential strategy in cholangiocarcinoma. *Transplantation.* **76**: 1003–1006.
37. Kawai T., A.B. Cosimi, T.R. Spitzer, N. Tolkoff-Rubin, M. Suthanthiran, S.L. Saidman, J. Shaffer, F.I. Preffer, R. Ding, V. Sharma, J.A. Fishman, B. Dey, D.S. Ko, M. Hertl, N.B. Goes, W. Wong, W.W. Williams, Jr., R.B. Colvin, M. Sykes, D.H. Sachs (2008). HLA-mismatched renal transplantation without maintenance immunosuppression. *N. Engl. J. Med.* **358**: 353–361.
38. Sayegh M.H., N.A. Fine, J.L. Smith, H.G. Rennke, E.L. Milford, N.L. Tilney (1991). Immunologic tolerance to renal allografts after bone marrow transplants from the same donors. *Ann. Intern. Med.* **114**: 954–955.
39. Svendsen U.G., S. Aggestrup, C. Heilmann, N. Jacobsen, C. Koch, B. Larsen, A. Svejgaard, B. Thisted, G. Petterson (1995). Transplantation of a lobe of lung from mother to child following previous transplantation with maternal bone marrow. *Eur. Respir. J.* **8**: 334–337.
40. Flores K.G., J. Li, G.D. Sempowski, B.F. Haynes, L.P. Hale (1999). Analysis of the human thymic perivascular space during aging. *J. Clin. Invest.* **104**: 1031–1039.
41. Liang Y., G. Van Zant, S.J. Szilvassy (2005). Effects of aging on the homing and engraftment of murine hematopoietic stem and progenitor cells. *Blood.* **106**: 1479–1487.
42. Rossi D.J., D. Bryder, J.M. Zahn, H. Ahlenius, R. Sonu, A.J. Wagers, I.L. Weissman (2005). Cell intrinsic alterations underlie hematopoietic stem cell aging. *Proc. Natl. Acad. Sci. USA.* **102**: 9194–9199.
43. Scadden D.T. (2006). The stem-cell niche as an entity of action. *Nature.* **441**: 1075–1079.
44. Stephan R.P., C.R. Reilly, P.L. Witte (1998). Impaired ability of bone marrow stromal cells to support B-lymphopoiesis with age. *Blood.* **91**: 75–88.
45. Tsuboi I., K. Morimoto, Y. Hirabayashi, G.X. Li, S. Aizawa, K.J. Mori, J. Kanno, T. Inoue (2004). Senescent B lymphopoiesis is balanced in suppressive homeostasis: decrease in interleukin-7 and

transforming growth factor-beta levels in stromal cells of senescence-accelerated mice. *Exp. Biol. Med. (Maywood)* **229**: 494–502.

46. Xing Z., M.A. Ryan, D. Daria, K.J. Nattamai, G. Van Zant, L. Wang, Y. Zheng, H. Geiger (2006). Increased hematopoietic stem cell mobilisation in aged mice. *Blood.* **108**: 2190–2197.
47. Yilmaz O.H., M.J. Kiel, S.J. Morrison (2006). SLAM family markers are conserved among hematopoietic stem cells from old and reconstituted mice and markedly increase their purity. *Blood.* **107**: 924–930.
48. Adams G.B., R.P. Martin, I.R. Alley, K.T. Chabner, K.S. Cohen, L.M. Calvi, H.M. Kronenberg, D.T. Scadden (2007). Therapeutic targeting of a stem cell niche. *Nat. Biotechnol.* **25**: 238–243.
49. Chidgey A.P., D. Layton, A. Trounson, R.L. Boyd (2008). Tolerance strategies for stem-cell-based therapies. *Nature.* **453**: 330–337.
50. van den Brink M.R., O. Alpdogan, R.L. Boyd (2004). Strategies to enhance T-cell reconstitution in immunocompromised patients. *Nat. Rev. Immunol.* **4**: 856–867.
51. Zakrzewski J.L., A.A. Kochman, S.X. Lu, T.H. Terwey, T.D. Kim, V.M. Hubbard, S.J. Muriglan, D. Suh, O.M. Smith, J. Grubin, N. Patel, A. Chow, J. Cabrera-Perez, R. Radhakrishnan, A. Diab, M.A. Perales, G. Rizzuto, E. Menet, E.G. Pamer, G. Heller, J.C. Zuniga-Pflucker, O. Alpdogan, M.R. van den Brink (2006). Adoptive transfer of T-cell precursors enhances T-cell reconstitution after allogeneic hematopoietic stem cell transplantation. *Nat. Med.* **12**: 1039–1047.
52. Steiner D., N. Brunicki, B.R. Blazar, E. Bachar-Lustig, Y. Reisner (2006). Tolerance induction by third-party "off-the-shelf" CD4+CD25+ Treg cells. *Exp. Hematol.* **34**: 66–71.
53. Tu W., Y.L. Lau, J. Zheng, Y. Liu, P.L. Chan, H. Mao, K. Dionis, P. Schneider, D.B. Lewis (2008). Efficient generation of human alloantigen-specific CD4+ regulatory T cells from naive precursors by CD40-activated B cells. *Blood.* **112**: 2554–2562.
54. Zhang X., M. Li, D. Lian, X. Zheng, Z.X. Zhang, T.E. Ichim, X. Xia, X. Huang, C. Vladau, M. Suzuki, B. Garcia, A.M. Jevnikar, W.P. Min (2008). Generation of therapeutic dendritic cells and regulatory T cells for preventing allogeneic cardiac graft rejection. *Clin. Immunol.* **127**: 313–321.
55. Goldberg G.L., O. Alpdogan, S.J. Muriglan, M.V. Hammett, M.K. Milton, J.M. Eng, V.M. Hubbard, A. Kochman, L.M. Willis, A.S. Greenberg, K.H. Tjoe, J.S. Sutherland, A. Chidgey, M.R. van den Brink, R.L. Boyd (2007). Enhanced immune reconstitution by sex steroid ablation following allogeneic hemopoietic stem cell transplantation. *J. Immunol.* **178**: 7473–7484.
56. Goldberg G.L., J. Dudakov, J.J. Reiseger, N. Seach, T. Ueno, K. Vlahos, M.V. Hammett, L. Young, T.S.P. Heng, R.L. Boyd, A.P. Chidgey (2009). Sex steroid ablation enhances immune reconstitution following cytotoxic antineoplastic therapy in young mice. *J. Pathol.*
57. Goldberg G.L., J.S. Sutherland, M.V. Hammet, M.K. Milton, T.S. Heng, A.P. Chidgey, R.L. Boyd (2005). Sex steroid ablation enhances lymphoid recovery following autologous hematopoietic stem cell transplantation. *Transplantation.* **80**: 1604–1613.
58. Greenstein B.D., F.T. Fitzpatrick, M.D. Kendall, M.J. Wheeler (1987). Regeneration of the thymus in old male rats treated with a stable analogue of LHRH. *J Endocrinol.* **112**: 345–350.
59. Marchetti B., V. Guarcello, M.C. Morale, G. Bartoloni, Z. Farinella, S. Cordaro, U. Scapagnini (1989). Luteinizing hormone-releasing hormone-binding sites in the rat thymus: characteristics and biological function. *Endocrinology.* **125**: 1025–1036.
60. Sutherland J.S., G.L. Goldberg, M.V. Hammett, A.P. Uldrich, S.P. Berzins, T.S. Heng, B.R. Blazar, J.L. Millar, M.A. Malin, A.P. Chidgey, R.L. Boyd (2005). Activation of thymic regeneration in mice and humans following androgen blockade. *J Immunol.* **175**: 2741–2753.

61. Sutherland J.S., L. Spyroglou, J.L. Muirhead, T.S. Heng, A. Prieto-Hinojosa, H.M. Prince, A.P. Chidgey, A.P. Schwarer, R.L. Boyd (2008). Enhanced Immune System Regeneration in Humans Following Allogeneic or Autologous Hemopoietic Stem Cell Transplantation by Temporary Sex Steroid Blockade. *Clin Cancer Res.* **14**: 1138–1149.
62. Chidgey A., J. Dudakov, N. Seach, R. Boyd (2007). Impact of niche aging on thymic regeneration and immune reconstitution. *Semin Immunol.* **19**: 331–340.
63. Nakagawa M., M. Koyanagi, K. Tanabe, K. Takahashi, T. Ichisaka, T. Aoi, K. Okita, Y. Mochiduki, N. Takizawa, S. Yamanaka (2008). Generation of induced pluripotent stem cells without Myc from mouse and human fibroblasts. *Nat. Biotechnol.* **26**: 101–106.
64. Park I.H., R. Zhao, J.A. West, A. Yabuuchi, H. Huo, T.A. Ince, P.H. Lerou, M.W. Lensch, G.Q. Daley (2008). Reprogramming of human somatic cells to pluripotency with defined factors. *Nature.* **451**: 141–146.
65. Takahashi K., K. Tanabe, M. Ohnuki, M. Narita, T. Ichisaka, K. Tomoda, S. Yamanaka (2007). Induction of pluripotent stem cells from adult human fibroblasts by defined factors. *Cell.* **131**: 861–872.
66. Yu J., M.A. Vodyanik, K. Smuga-Otto, J. Antosiewicz-Bourget, J.L. Frane, S. Tian, J. Nie, G.A. Jonsdottir, V. Ruotti, R. Stewart, Slukvin, II, J.A. Thomson (2007). Induced pluripotent stem cell lines derived from human somatic cells. *Science.* **318**: 1917–1920.
67. Braga L.M., K. Rosa, B. Rodrigues, C. Malfitano, M. Camassola, P. Chagastelles, S. Lacchini, P. Fiorino, K. De Angelis, B.D. Schaan, M.C. Irigoyen, N.B. Nardi (2008). Systemic delivery of adult stem cells improves cardiac function in spontaneously hypertensive rats. *Clin. Exp. Pharmacol. Physiol.* **35**: 113–119.
68. Endres M., K. Neumann, T. Haupl, C. Erggelet, J. Ringe, M. Sittinger, C. Kaps (2007). Synovial fluid recruits human mesenchymal progenitors from subchondral spongious bone marrow. *J. Orthop. Res.* **25**: 1299–1307.
69. Hatch H.M., D. Zheng, M.L. Jorgensen, B.E. Petersen (2002). SDF-1alpha/CXCR4: a mechanism for hepatic oval cell activation and bone marrow stem cell recruitment to the injured liver of rats. *Cloning Stem Cells.* **4**: 339–351.
70. Kopp H.G., C.A. Ramos, S. Rafii (2006). Contribution of endothelial progenitors and proangiogenic hematopoietic cells to vascularisation of tumour and ischemic tissue. Curr Opin Hematol **13**: 175–181.
71. Mimeault M., S.K. Batra (2008). Recent progress on tissue-resident adult stem cell biology and their therapeutic implications. *Stem Cell Rev.* **4**: 27–49.
72. Brack A.S., I.M. Conboy, M.J. Conboy, J. Shen, T.A. Rando (2008). A temporal switch from notch to Wnt signaling in muscle stem cells is necessary for normal adult myogenesis. *Cell Stem Cell.* **2**: 50–59.
73. Gurtner G.C., S. Werner, Y. Barrandon, M.T. Longaker (2008). Wound repair and regeneration. *Nature.* **453**: 314–321.
74. Sun X., X. Fu, Z. Sheng (2007). Cutaneous stem cells: something new and something borrowed. *Wound Repair Regen.* **15**: 775–785.
75. Hall B., J. Dembinski, A.K. Sasser, M. Studeny, M. Andreeff, F. Marini (2007). Mesenchymal stem cells in cancer: tumour-associated fibroblasts and cell-based delivery vehicles. *Int. J. Hematol.* **86**: 8–16.
76. Kucerova L., V. Altanerova, M. Matuskova, S. Tyciakova, C. Altaner (2007). Adipose tissue-derived human mesenchymal stem cells mediated prodrug cancer gene therapy. *Cancer Res.* **67**: 6304–6313.

77. Mapara K.Y., C.B. Stevenson, R.C. Thompson, M. Ehtesham (2007). Stem cells as vehicles for the treatment of brain cancer. *Neurosurg Clin. N. Am.* **18**: 71–80, ix.

78. Yu J.J., X. Sun, X. Yuan, J.W. Lee, E.Y. Snyder, J.S. Yu (2006). Immunomodulatory neural stem cells for brain tumour therapy. *Expert. Opin. Biol. Ther.* **6**: 1255–1262.

79. Jiang Y., B.N. Jahagirdar, R.L. Reinhardt, R.E. Schwartz, C.D. Keene, X.R. Ortiz-Gonzalez, M. Reyes, T. Lenvik, T. Lund, M. Blackstad, J. Du, S. Aldrich, A. Lisberg, W.C. Low, D.A. Largaespada, C.M. Verfaillie (2002). Pluripotency of mesenchymal stem cells derived from adult marrow. *Nature.* **418**: 41–49.

80. Wang G., B.A. Bunnell, R.G. Painter, B.C. Quiniones, S. Tom, N.A. Lanson, Jr., J.L. Spees, D. Bertucci, A. Peister, D.J. Weiss, V.G. Valentine, D.J. Prockop, J.K. Kolls (2005). Adult stem cells from bone marrow stroma differentiate into airway epithelial cells: potential therapy for cystic fibrosis. *Proc. Natl. Acad. Sci. USA.* **102**: 186–191.

81. Kopen G.C., D.J. Prockop, D.G. Phinney (1999). Marrow stromal cells migrate throughout forebrain and cerebellum, and they differentiate into astrocytes after injection into neonatal mouse brains. *Proc. Natl. Acad. Sci. USA.* **96**: 10711–10716.

82. Brooke G., M. Cook, C. Blair, R. Han, C. Heazlewood, B. Jones, M. Kambouris, K. Kollar, S. McTaggart, R. Pelekanos, A. Rice, T. Rossetti, K. Atkinson (2007). Therapeutic applications of mesenchymal stromal cells. *Sem. Cell. Dev. Biol.* **18**: 846–858.

83. Lee P.H., J.W. Kim, O.Y. Bang, Y.H. Ahn, I.S. Joo, K. Huh (2007). Autologous Mesenchymal Stem Cell Therapy Delays the Progression of Neurological Deficits in Patients With Multiple System Atrophy. *Clin. Pharmacol. Ther.* **83**: 723–730.

84. Blanc K. Le, O. Ringden (2007). Immunomodulation by mesenchymal stem cells and clinical experience. *J. Intern Med.* **262**: 509–525.

85. Fouillard L., A. Chapel, D. Bories, S. Bouchet, J.M. Costa, H. Rouard, P. Herve, P. Gourmelon, D. Thierry, M. Lopez, N.C. Gorin (2007). Infusion of allogeneic-related HLA mismatched mesenchymal stem cells for the treatment of incomplete engraftment following autologous haematopoietic stem cell transplantation. *Leukemia.* **21**: 568–570.

86. Koc O.N., S.L. Gerson, B.W. Cooper, S.M. Dyhouse, S.E. Haynesworth, A.I. Caplan, H.M. Lazarus (2000). Rapid hematopoietic recovery after coinfusion of autologous-blood stem cells and culture-expanded marrow mesenchymal stem cells in advanced breast cancer patients receiving high-dose chamotherapy. *J. Clin. Oncol.* **18**: 307–316.

87. Lazarus H.M., O.N. Koc, S.M. Devine, P. Curtin, R.T. Maziarz, H.K. Holland, E.J. Shpall, P. McCarthy, K. Atkinson, B.W. Cooper, S.L. Gerson, M.J. Laughlin, F.R. Loberiza, Jr., A.B. Moseley, A. Bacigalupo (2005). Cotransplantation of HLA-identical sibling culture-expanded mesenchymal stem cells and hematopoietic stem cells in hematologic malignancy patients. *Biol. Blood. Marrow. Transplant.* **11**: 389–398.

88. Blanc K. Le, F. Frassoni, L. Ball, F. Locatelli, H. Roelofs, I. Lewis, E. Lanino, B. Sundberg, M.E. Bernardo, M. Remberger, G. Dini, R.M. Egeler, A. Bacigalupo, W. Fibbe, O. Ringden (2008). Mesenchymal stem cells for treatment of steroid-resistant, severe, acute graft-versus-host disease: A phase II study. *Lancet.* **371**: 1579–1586.

89. Blanc K. Le, I. Rasmusson, B. Sundberg, C. Gotherstrom, M. Hassan, M. Uzunel, O. Ringden (2004). Treatment of severe acute graft-versus-host disease with third party haploidentical mesenchymal stem cells. *Lancet.* **363**: 1439–1441.

90. Blanc K. Le , H. Samuelsson, B. Gustafsson, M. Remberger, B. Sundberg, J. Arvidson, P. Ljungman, H. Lonnies, S. Nava, O. Ringden (2007). Transplantation of mesenchymal stem cells to enhance engraftment of hematopoietic stem cells. *Leukemia.* **21**: 1733–1738.
91. Lee S.T., J.H. Jang, J.W. Cheong, J.S. Kim, H.Y. Maemg, J.S. Hahn, Y.W. Ko, Y.H. Min (2002). Treatment of high-risk acute myelogenous leukaemia by myeloablative chemoradiotherapy followed by co-infusion of T cell-depleted haematopoietic stem cells and culture-expanded marrow mesenchymal stem cells from a related donor with one fully mismatched human leucocyte antigen haplotype. *Br. J. Haematol.* **118**: 1128–1131.
92. Ringden O., M. Uzunel, I. Rasmusson, M. Remberger, B. Sundberg, H. Lonnies, H.U. Marschall, A. Dlugosz, A. Szakos, Z. Hassan, B. Omazic, J. Aschan, L. Barkholt, K. Le Blanc (2006). Mesenchymal stem cells for treatment of therapy-resistant graft-versus-host disease. *Transplantation.* 81: 1390–1397.
93. Sippel K.C., M.J. J., F.C. S. (2001). Amniotic membrane surgery. *Curr Opin Ophthalmol.* **12**: 269–281.
94. Ilancheran S., A. Michalska, G. Peh, E.M. Wallace, M. Pera, U. Manuelpillai (2007). Stem cells derived from human Foetal membranes display multilineage differentiation potential. *Biol. Reprod.* **77**: 577–588.
95. Miki T., T. Lehmann, H. Cai, D.B. Stolz, S.C. Strom (2005). Stem cell characteristics of amniotic epithelial cells. *Stem Cells.* **23**: 1549–1559.
96. Sakuragawa N., S. Enosawa, T. Ishii, R. Thangavel, T. Tashiro, T. Okuyama, S. Suzuki (2000). Human amniotic epithelial cells are promising transgene carriers for allogeneic cell transplantation into liver. *J. Hum. Genet.* **45**: 171–176.
97. Takashima S., H. Ise, P. Zhao, T. Akaike, T. Nikaido (2004). Human amniotic epithelial cells possess hepatocyte-like characteristics and functions. *Cell Struct. Funct.* **29**: 73–84.
98. Elwan M.A., N. Sakuragawa (1997). Evidence for synthesis and release of catecholamines by human amniotic epithelial cells. *Neuroreport.* **8**: 3435–3438.
99. Kakishita K., M.A. Elwan, N. Nakao, T. Itakura, N. Sakuragawa (2000). Human amniotic epithelial cells produce dopamine and survive after implantation into the striatum of a rat model of Parkinson's disease: A potential source of donor for transplantation therapy. *Exp. Neurol.* **165**: 27–34.
100. Kakishita K., N. Nakao, N. Sakuragawa, T. Itakura (2003). Implantation of human amniotic epithelial cells prevents the degeneration of nigral dopamine neurons in rats with 6-hydroxydopamine lesions. *Brain. Res.* **980**: 48–56.
101. Sakuragawa N., H. Misawa, K. Ohsugi, K. Kakishita, T. Ishii, R. Thangavel, J. Tohyama, M. Elwan, Y. Yokoyama, O. Okuda, H. Arai, I. Ogino, K. Sato (1997). Evidence for active acetylcholine metabolism in human amniotic epithelial cells: Applicable to intracerebral allografting for neurologic disease. *Neurosci. Lett.* **232**: 53–56.
102. Banas R.A., C. Trumpower, C. Bentlejewski, V. Marshall, G. Sing, A. Zeevi (2008). Immunogenicity and immunomodulatory effects of amnion-derived multipotent progenitor cells. *Hum. Immunol.* **69**: 321–328.
103. Fang B., Y. Song, Q. Lin, Y. Zhang, Y. Cao, R.C. Zhao, Y. Ma (2007). Human adipose tissue-derived mesenchymal stromal cells as salvage therapy for treatment of severe refractory acute graft-vs.-host disease in two children. *Pediatr. Transplant.* **11**: 814–817.
104. Ball L., R. Bredius, A. Lankester, J. Schweizer, M. van den Heuvel-Eibrink, H. Escher, W. Fibbe, M. Egeler (2008). Third party mesenchymal stromal cell infusions fail to induce tissue repair despite