Regenerative Medicine: Challenges and Perspectives for Successful Therapies

S. Viswanathan and C. Joshi

Abstract Regenerative Medicine (RM) has the promise to revolutionize the treatment of many debilitating diseases for which the current therapies are inadequate. To realize the full potential of RM, a pragmatic approach needs to be taken by all stakeholders keeping in mind the lessons learnt from recombinant protein manufacturing, gene therapy trials, etc., to develop novel service delivery models for economic viability and regulatory processes in the absence of long-term data. In this chapter, we focus on the three main drivers of RM field and discuss the potential pitfalls and possible ways to mitigate them in order to move the field closer to clinical implementation.

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Current Topics in Microbiology and Immunology (2013) 367: 101–112
DOI: 10.1007/82_2012_290

Published Online: 4 December 2012

1 Introduction

As the median age of the population shifts higher and as improvements in clinical and pharmaceutical management push the lifespan longer, debilitating degenerative diseases are gaining the spotlight as the new medical challenge that needs to be addressed in ever greater demographic. In this context, Regenerative Medicine (RM) promises to be the next leap in innovating the standard of care, and possibly offering curative solutions to many degenerative conditions.

In this chapter, we will focus on three main aspects of RM, cell-based therapies, tissue engineering and harnessing the regenerative capacity of endogenous organs, and review the recent progress and major obstacles in each.

2 RM and Cell-Based Therapies

RM and stem cells are intricately linked. Stem cells provide the nodal points for obtaining a variety of cell subtypes with specialized functions—the very functions that RM aims to restore such as insulin producing beta cell or, in case of cyto-immunotherapy, novel functions such as targeted anti-tumor effect. Starting from the discovery of hematopoietic stem cells (HSC) (Becker et al. 1963), and building on the knowledge gained from decades of research on HSC, the discoveries of other somatic tissue adult stem cell-like cells with restricted potential such as mesenchymal stromal cells (MSC) (Pittenger et al. 1999), muscle satellite cells (Seale and Rudnicki 2000), and neuronal stem cells (NSC) (Reynolds and Weiss 1992) have incrementally moved the RM field forward. However, the discovery of embryonic stem cells (ESC) (Thomson et al. 1998) has been seminal in augmenting interest in this area by several orders of magnitude. This is because ESCs afforded access to previously unavailable compartments such as cardio vascular and mesendo-dermal derivatives (such as beta cells) owing to their pluripotency. Also, this cell type in principle, removes the obstacle of obtaining stem cells in sufficient quantities to remedy a particular disease, as it would truly be used for cell-replacement therapy. However, ESCs also brought with them ethical controversies and technological limitations. The field of RM got a second enormous boost following the discovery of methods to "induce" multi or pluripotency in post-mitotic somatic cells—by generation of induced pluripotent cells (iPS) (Takahashi and Yamanaka 2006). Through iPS cells, it is now possible to obtain disease-specific and patient-specific stem cells, their differentiated progeny and model the disease progression, discover appropriate intervening actions, achieve a desirable number of target cells for administration, and even mitigate immunorejection without using embryonic tissue and thereby eliminate ethical concerns (Robinton and Daley 2012). Advances in this area are also being made with "direct" reprogramming where it may be feasible to reprogram one mature phenotype to another without transmitting to a less mature state (Davis et al. 1987;

Vierbuchen et al. 2010; Pang et al. 2011; Huang et al. 2011), although it is unclear how similar this re-programming process is to normal development.

2.1 Recent Progress in Cell-Based Therapies

Considerable effort and research investment is being made to harness the potential of cell-based therapies in almost all physiological and pathological conditions. There are over 5,000 clinical trials ongoing globally with various stem cells, and a similar number or more in the preclinical translational stage of research (www.clinicaltrials.org; (Culme-Seymour et al. 2000)). Despite this tremendous interest and progress, only a handful of cell-based therapies are commercially available including Osiris's Prochymal for pediatric Graft-versus-Host Disease (GvHD) in Canada and New Zealand (2012), Dendreon's Provenge for metastatic castrate-resistant prostrate cancer (2010), Genzyme's Carticel (1997) for articular cartilage injuries, TETEC's Novocart® (for joint cartilage), etc. In the pipeline though are a few companies employing adult stem cells in late phase clinical trials such as Aastrom's Ixmyelocel-T for Critical Limb Ischemia, Mesoblast's Mesenchymal Precursor Cells for Type II Diabetes, Recent Acute Myocardial Infarction, Heart Failure, etc.; positive outcomes in Phase II and Phase III trials are needed to enable market approval and justify the commercialization of these products. Others such as Geron's Phase I trial using hESC-derived oligodendrocytes have stopped investigations because of economic non-viability. There are a number of safety, efficacy, manufacturing, regulatory, and economic hurdles that need to be overcome to enable the successful commercialization of stem cell-based therapeutics.

Considerable effort has also been made into translating the promise of gene therapy. Over the past decade a number of clinical studies have provided proof of concept that genetically modified hematopoietic stem and progenitor cells can be used to treat metabolic disorders and monogenic diseases (Cavazzana-Calvo et al. 2010; Cartier et al. 2009). Recently, allogenic stem cell transplantation into an HIV-positive individual was shown to result in long-term cure demonstrating the feasibility and potency of genetic transfer using modified stem cells (Hutter and Zaia 2011). Adoptive immunotherapy using engineered T-cells has largely been shown to be safe in over 180 patients (Cruz et al. 2010) with compelling evidence in the case of melanoma (Rosenberg et al. 2011). T-cells engineered with chimeric antigen receptors (CARs) are also gaining favor especially in treating B-cell neoplasias (Brentjens et al. 2011; Porter et al. 2011).

Regenerative medicine approaches to understand disease progression in integration with high-throughput screening platforms has resulted in application of cell therapy tools in drug discovery programs. The ability to model complex diseases through use of stem cell-based assays and in vitro mini-tissue architectures has allowed the interrogation of disease states and their manipulation by pharmacological agents and small molecules. Chemical screens, libraries of previously

purposed small molecules are all being screened in the hope of finding the next "hit" such as the anti-microbial agent, tigecycline and its ability to selectively inhibit leukemic stem cells (Skrtic et al. 2011). Another promising drug repurposing has been shown with Zeluton, an approved drug for asthma that inhibits activity of Alox5 gene product, which can also effectively block chronic myeloid leukemia (CML) stem cells that cause gleevac-resistant CML relapse. Other molecules such as inhibitors of HSP90 and hedgehog pathways are also being screened for this purpose (Chen et al. 2010). Commercially produced hESC-derived cardiomyocytes and hepatocytes have been in extensive use for toxicology screening (Jensen et al. 2009; Sartipy and Bjorquist 2011). Disease modeling through pluripotent stem cell-derived populations complements direct cell-or tissue-replacement therapeutics, and is getting attention from pharmaceutical companies like GE and GSK (Ebert et al. 2012).

As more commercial interest is generated and investments in cell-based therapeutics areas grow, the field will evolve towards more translational aspects of a therapeutic requirements such as product safety, quality assurance, scale-up and manufacturing, and reproducibility, delivery and dosage formats (Carmen et al. 2012). There are some significant challenges to overcome in this area as described below.

2.2 Challenges to Overcome for Cell-Based Therapies

2.2.1 Manufacturing

Stem cells, unlike tumor cell lines, cannot be easily cultured in large batches as their functionality and quality are highly susceptible to cell culture conditions. Cell doses per patient can vary depending on the type and application, but anywhere from 10^6 to 10^{10} cells may be needed for a standard dose. To treat 1,000 patients a year, correspondingly 10^9 to 10^{13} cells would be required. The current technologies of stem cell culture allow for production of a fraction of that number. Obtaining specialized, functional, terminally differentiated cells from stem cells in that quantity remains a serious hurdle. New technologies for scalable bioreactor culture and processing are needed to derive enough target cells continuously in an industrial setting (Zweigerdt 2009).

Manufacturing of these cells under current Good Manufacturing Practice (cGMP) conditions poses additional challenges requiring closed systems, USP-grade reagents, highly screened and quality controlled raw materials, and highly standardized manipulation steps. The traditional fill- and finish-model of biopharmaceutical manufacturing is not applicable to a cellular system with limited viability and varying functionality. A separation process to isolate, maintain, and purify the cell product, cell expansion and manipulation, and storage and delivery system that retains viability and functionality of the product needs to be developed and validated at appropriate scales for each therapeutic product (Amos et al. 2012).

There are a lot of lessons to learn from production of cells for recombinant proteins and vaccines including aspects of biotherapeutic protein supply chain such as standardized raw materials (cell banks), cold chain storage and distribution, and lot validation. However the relatively shorter shelf-life of the cell-therapy product poses additional challenges often requiring lot-release and validation to be performed post-shipping. Autologous cell therapy products pose additional challenges such as variable raw materials and variable end product with wide tolerances in potency as well as on-site manufacturing, validation, supply, and delivery facilities. Even if technologies are licensed and stringent SOPs are in place, local variations in cell manipulation during manufacture and delivery to patients may result in heterogeneous success rates across centers.

To implement genetically modified cells as standard-of-care, larger Phase II and III trials need to be undertaken and the challenges of manufacturing GMP-grade stem cells apply to this field as well. Additionally, there are the challenges of manufacturing, purifying, and concentrating clinical grade viral vectors, although alternatives such as nanoparticles (Hosseinkhani and Tabata 2006) and lipid-based complexes (Fenske et al. 2008) are beginning to emerge.

2.2.2 Regulation

Regulatory and marketing authorities rely heavily on historical data and animal studies for safety and efficacy results. In the case of most cell-based products, long-term safety and efficacy data are not available in animal models or in humans. Thus initiation of Phase I trials is often challenging for both regulators and sponsors in this nascent field, as the properties of the cells, their mechanism of action, their biodistribution, and long-term safety effects are all usually not fully-defined in a preclinical setting. Clinical studies are often undertaken in patients who are not in the ideal disease-progression stage for efficacious cell-therapy investigations casting doubts on the utility of such treatments when inevitably mixed results are obtained in a Phase I/II setting. Increased dialogue and interaction between the regulators and sponsors will allow this field to advance. There is promising evidence that this is already happening; the FDA has been open to dialoguing with sponsors and investigators prior to initiation of clinical trials via their pre-Investigative New Drug (IND) and even pre–pre-IND meetings. Additional guidelines specific to various tissues have been developed and put out by the FDA. Out-of-the-box thinking has been also demonstrated by reviewers at Health Canada who provided market approval for pediatric use of Prochymal to treat GvHD in the absence of complete efficacy data, and compromising by allowing Phase IV studies to collect such data.

It will be up to the scientists, regulators, industry, and medical professionals to manage risks and expectations from cell therapies without hype but also without risk-averse bias since the emerging experimental therapies are most likely to provide variably efficacy data and lack the precision that years of pharmaceutical experience has provided.

2.2.3 Economics

Cell-based therapies are a highly specialized branch of medicine with multiple steps requiring continuous integration with healthcare providers, laboratory staff, cell manufacturing facilities, and administrators. Autologous therapies are typically not suited for acute conditions; off-the shelf, allogeneic products may work more broadly; however, this requires better understanding of the long-term implications of culture-expanded cells. The normal service delivery model of biopharmaceutical manufacturing may also not apply, except for off-the-shelf allogeneic products. Cell-based therapies may require healthcare facility to house special infrastructure, for example, cGMP facilities or clean-rooms, or additional validated laboratory equipment within an operating theater to provide such therapies to patients. A different delivery model, knowledgeable personnel, infrastructure, and equipment are needed to support this process. Insurance companies and governments are not necessarily ready to assume these costs, especially when efficacy and long-term benefits are still being answered in clinical trials. To support these therapeutics and to make them economically viable, a new service delivery model system would be required (Luijten et al. 2012). Public-Private partnership models currently in use in the Netherlands may provide a blueprint for a system where cell manufacturing is carried out by private companies and the administering of the product, follow-up for safety, efficacy, and clinical oversight are performed at the publicly-funded healthcare institutions. In an environment where healthcare costs are ballooning, individual subscriptions to healthcare insurance plans may need to be enforced either through indirect taxation or by way of user fees. It may require innovative political approaches to educate the public and obtain sufficient participation from all stakeholders.

3 Tissue Engineering and RM

Tissue engineering has a number of applications ranging from traditional replacement and repair of structural tissues such as skin, bone, cartilage to engineering complex organs (liver, pancreas, kidney, heart, etc.) including engineered blood vessels (Miller et al. 2012), and bioprosthetic heart valves (porcine, bovine, cadaveric, or pulmonary-to-aortic autografts) (Mendelson and Schoen 2006) to providing research tools to understand tissue functioning. For example, using the latest advances in microfluidics technology, it is now possible to study fluid dynamics and blood cell interactions using a 3-D microvascular network on a chip (Zheng et al 2012).

Tissue engineering approaches are providing novel solutions to cell-and tissue-repair and replacement issues. For example, in vivo tissue-integration of iPSC-derived cardiomyocytes may prove difficult, however, use of emerging technologies such as 3D-angiogenic printing (Miller et al. 2012) and biomaterial scaffolds allows

one to mimic the in vivo niche microenvironment and enable creation of cardiac-patches that may more easily integrate with the host tissue (Dengler et al. 2011).

Examples of commercial success of tissue engineered products include Organogenesis' Apligraf (1998) which combines a collagen matrix with fibroblasts and keratinocytes, Genzyme's Epicel (1998) which uses cultured autologous patient keratinocytes to form sheets that are stapled onto petrolatum gauze backing, Tissue Regenix's dCELL® Vascular Patch (2010), Cytograft's Lifeline, a tissue engineered blood vessel that has approval for autologous use in Germany (2009), and others.

3.1 Challenges to Tissue Engineering

3.1.1 Biomaterials

Different types of biomaterials ranging from natural, synthetic to composite, unmodified to modified chemically or physically and available in a variety of forms, injectable or non-injectable provide not just structural cues, but also microenvironmental cues to truly modulate surrounding cells and tissue (Davis et al. 2005). Indeed the use of microfabricated arrays of stem cell regulatory factors and extracellular matrix (ECM) components has been used to demonstrate the complex network of regulatory signals involved in self-renewal and differentiation of neural stem cells (Soen et al. 2006). Identifying, engineering, and optimizing specific biomaterials for appropriate end-uses remains a challenge, especially as multiple parameters from stability, biocompatibility, release of growth factors, physical support, etc., need to be considered and appropriately configured for specific tissue uses.

Biomaterials can also be designed to respond to variations in microenvironmental acidity, temperature, sheer stress, oxygenation, or enzyme levels (Stoop 2008; Rosso et al. 2005; Williams 2005) and thus provide directional and sequential release of growth factors resulting in appropriate spatio-temporal gradients. Smart biomaterials, although in their infancy, can therefore be developed along with advances in micromolding, laser photolithography, and microfluidic devices to create complex, controlled networks for drug/growth factor delivery.

3.1.2 Vascularization of Engineered Tissue

In addition to having biocompatible and smart biomaterials, it is important to have vascular networks as the transport of oxygen, nutrient, and waste is currently a major challenge in the field of tissue engineering. Strategies to induce network of blood vessels in vivo (Laschke et al. 2006; Lovett et al. 2009) or implanting pre-vascularized scaffolds will be critical to successful grafting, integration, survival, and functioning of these engineered tissue grafts, especially complex tissues such

as kidney, heart, and liver. There are a number of strategies postulated including controlled and local release of angiogenic factors to promote neovascularization (Silva and Mooney 2007), but this still remains a major hurdle in designing and integrating viable scaffolds and constructs in vivo.

In addition, managing immune reaction to engineered tissue grafts requires separate strategies ranging from reduction of the graft immunogenicity and immunosuppressive regimes with mesenchymal stromal cells or donor-derived immunosuppressive antigen-presenting cells, and poses separate technical and regulatory issues that cannot be overlooked.

4 Regenerative Capacity of Endogenous Organs

The idea of recruiting resident tissue stem cell populations for effecting repair in injury or degenerative conditions is not new, but the widespread existence of such cells and the provoking concept of using bioactive molecules to recruit and engage such cells in an endogenous repair process are still nascent. Proof-of-concept has already been demonstrated by the use of erythropoietin to enhance blood cell formation, Granulocyte-Colony Stimulating Factor (G-CSF) for mobilization of hematopoietic precursors, and Bone Morphogenetic Protein (BMP)-2 which promotes osteogenesis from mesenchymal precursors. Several high-profile chemical screens have been published on several stem cell types, including HSCs, which identified soluble factors that inhibit ligand-induced signaling by aryl hydrocarbon receptor (AhR), and thus promote expansion of mobilized peripheral blood and umbilical cord blood (Boitano et al. 2010). Other small molecules that have been identified include a peroxisome proliferator-activated receptor-gamma (PPAR-g) inhibitor bipshenol A diglycidyl ether which has been shown to accelerate hematopoietic engraftment (Naveiras et al. 2009), and parathyroid hormone (PTH) which has been shown to improve mobilization and engraftment during sequential cord blood transplantation, and is currently in clinical investigations (NCT00393380 and NCT00299780).

Use of growth factors or small molecules may be particularly effective as treatment strategies for a variety of extreme psychiatric disorders including schizophrenia, extreme depression (having failed even electroconvulsive therapy), etc., as signals that are implicated in normal stem cell maintenance such as brain-derived neural factor (BDNF) may be disrupted in mood disorders, and thus serve as targets for pharmacological-based intervention (Benninghoff 2009).

Despite the promise of using small molecules or growth factors to harness the endogenous potential of tissue resident stem cells, there are concerns regarding delivery and retention of these molecules in required concentrations, potential harmful systemic side effects, and potential tumorigenic safety concerns for highly active bioactive molecules that promote endogenous stem cell recruitment and proliferation (rev in. (Miller and Kaplan 2012)).

Proposed solutions include identifying small molecules or drugs with a demonstrable safety profile that can be re-purposed to target endogenousstem cells or perhaps their niche environment, which may be particularly relevant for targeting cancers (rev in. (Wagers 2012)). This has been shown to some extent with the use of G-CSF mobilization, which can reduce amyloid plaque deposition in the hippocampus and improve cognition in murine models of Alzheimer's disease, although, the pilot study suggested safety but questionable efficacy in humans (Sanchez-Ramos et al. 2012).

5 Next Steps

Unlike research and delivery timescales in cancer or infectious disease medicine, RM has come a long way rapidly, largely owing to the successes in stem cell science. This has led to inflated expectations from a rapidly growing demographic consisting of aging population and their care providers to deliver better quality of life through RM along with substantial media hype (Eisenstein 2012). Therein lies the challenge for the scientific and clinical community to translate the research knowledge rapidly and safely enough to meet this demand. In the absence of significant gains, stem cell clinics are sprouting globally and offering cures to desperate patients and families although in most cases the cells have not been experimentally tested, protocols are not scientifically or ethically reviewed by third parties, and there is no independent monitoring of patient safety and welfare.

Since RM is most likely to offer benefits to an aging population, emerging ideas about stem cells and aging need to be kept in mind. An accumulation of myeloid biased versus lymphoid-biased HSC with increasing age and expenditure of skeletal muscle stem cells in the aging niche underscore that both niche and stem cell populations may be adversely affected by aging (Baumann 2012; Muller-Sieburg et al. 2012), and this factor could limit the utility of at least autologous stem cells in the very population that stands to benefit the most.

The growth of personalized medicine by way of whole-genome sequencing may help in prognosticating patients who might benefit the most with RM-therapeutics versus those who may experience poor outcomes because of failure of therapy or unacceptable side-effects. This may further streamline creation of ratiometric formulae for cost/benefit, and thus make the risk acceptable to all stakeholders.

Measured solutions from multiple fields including researchers, engineers and manufacturers, regulators, and the business development side are needed to develop viable and profitable business and/or services models to translate largely personalized regenerative medicine concepts to routine clinical practice. Hard lessons learned from gene therapy failures (Wilson 2009) can be applied to regenerative medicine applications including the importance of adhering to the protocols, proper training, accreditation, and documentation involving the staff and therapeutic product preparation and administration, and avoiding conflicts of interest to ensure the field moves forward in a responsible manner. With these in

place, we can optimistically expect the field to make significant advances in providing novel therapeutic solutions to patients, and indeed become practice-changing over the next decade.

References

Amos PJ, Cagavi Bozkulak E, Qyang Y (2012) Methods of cell purification: a critical juncture for laboratory research and translational science. Cells Tissues Organs 195(1–2):26–40

Baumann K (2012) Stem cells: an ageing decline. Nat Rev Mol Cell Biol 13(11):681

Becker AJ, Mc CE, Till JE (1963) Cytological demonstration of the clonal nature of spleen colonies derived from transplanted mouse marrow cells. Nature 197:452–454

Benninghoff J (2009) Stem cell approaches in psychiatry–challenges and opportunities. Dialogues Clin Neurosci 11(4):397–404

Boitano AE, Wang J, Romeo R, Bouchez LC, Parker AE, Sutton SE et al (2010) Aryl hydrocarbon receptor antagonists promote the expansion of human hematopoietic stem cells. Science 329(5997):1345–1348

Brentjens RJ, Riviere I, Park JH, Davila ML, Wang X, Stefanski J et al (2011) Safety and persistence of adoptively transferred autologous CD19-targeted T cells in patients with relapsed or chemotherapy refractory B-cell leukemias. Blood 118(18):4817–4828

Carmen J, Burger SR, McCaman M, Rowley JA (2012) Developing assays to address identity, potency, purity and safety: cell characterization in cell therapy process development. Regen Med 7(1):85–100

Cartier N, Hacein-Bey-Abina S, Bartholomae CC, Veres G, Schmidt M, Kutschera I et al (2009) Hematopoietic stem cell gene therapy with a lentiviral vector in X-linked adrenoleukodystrophy. Science 326(5954):818–823

Cavazzana-Calvo M, Payen E, Negre O, Wang G, Hehir K, Fusil F et al (2010) Transfusion independence and HMGA2 activation after gene therapy of human beta-thalassaemia. Nature 467(7313):318–322

Chen Y, Peng C, Sullivan C, Li D, Li S (2010) Novel therapeutic agents against cancer stem cells of chronic myeloid leukemia. Anticancer Agents Med Chem 10(2):111–115

Cruz CR, Hanley PJ, Liu H, Torrano V, Lin YF, Arce JA et al (2010) Adverse events following infusion of T cells for adoptive immunotherapy: a 10-year experience. Cytotherapy 12(6):743–749

Culme-Seymour EJ, Davie NL, Brindley DA, Edwards-Parton S, Mason C (2012) A decade of cell therapy clinical trials (2000–2010). Regen Med 7(4):455–462

Davis RL, Weintraub H, Lassar AB (1987) Expression of a single transfected cDNA converts fibroblasts to myoblasts. Cell 51(6):987–1000

Davis ME, Hsieh PC, Grodzinsky AJ, Lee RT (2005) Custom design of the cardiac microenvironment with biomaterials. Circ Res 97(1):8–15

Dengler J, Song H, Thavandiran N, Masse S, Wood GA, Nanthakumar K et al (2011) Engineered heart tissue enables study of residual undifferentiated embryonic stem cell activity in a cardiac environment. Biotechnol Bioeng 108(3):704–719

Ebert AD, Liang P, Wu JC (2012) Induced pluripotent stem cells as a disease modeling and drug screening platform. J Cardiovasc Pharmacol 60(4):408–416

Eisenstein M (2012) Stem cells: don't believe the hype. Nature 484(7393):S5

Fenske DB, Chonn A, Cullis PR (2008) Liposomal nanomedicines: an emerging field. Toxicol Pathol 36(1):21–29

Hosseinkhani H, Tabata Y (2006) Self assembly of DNA nanoparticles with polycations for the delivery of genetic materials into cells. J Nanosci Nanotechnol 6(8):2320–2328

Huang P, He Z, Ji S, Sun H, Xiang D, Liu C et al (2011) Induction of functional hepatocyte-like cells from mouse fibroblasts by defined factors. Nature 475(7356):386–389

Hutter G, Zaia JA (2011) Allogeneic haematopoietic stem cell transplantation in patients with human immunodeficiency virus: the experiences of more than 25 years. Clin Exp Immunol 163(3):284–295

Jensen J, Hyllner J, Bjorquist P (2009) Human embryonic stem cell technologies and drug discovery. J Cell Physiol 219(3):513–519

Laschke MW, Harder Y, Amon M, Martin I, Farhadi J, Ring A et al (2006) Angiogenesis in tissue engineering: breathing life into constructed tissue substitutes. Tissue Eng 12(8):2093–2104

Lovett M, Lee K, Edwards A, Kaplan DL (2009) Vascularization strategies for tissue engineering. Tissue Eng Part B Rev 15(3):353–370

Luijten PR, van Dongen GA, Moonen CT, Storm G, Crommelin DJ (2012) Public-private partnerships in translational medicine: concepts and practical examples. J Control Release 161(2):416–421

Mendelson K, Schoen FJ (2006) Heart valve tissue engineering: concepts, approaches, progress, and challenges. Ann Biomed Eng 34(12):1799–1819

Miller FD, Kaplan DR (2012) Mobilizing endogenous stem cells for repair and regeneration: are we there yet? Cell Stem Cell 10(6):650–652

Miller JS, Stevens KR, Yang MT, Baker BM, Nguyen DH, Cohen DM et al (2012) Rapid casting of patterned vascular networks for perfusable engineered three-dimensional tissues. Nat Mater 11(9):768–774

Muller-Sieburg CE, Sieburg HB, Bernitz JM, Cattarossi G (2012) Stem cell heterogeneity: implications for aging and regenerative medicine. Blood 119(17):3900–3907

Naveiras O, Nardi V, Wenzel PL, Hauschka PV, Fahey F, Daley GQ (2009) Bone-marrow adipocytes as negative regulators of the haematopoietic microenvironment. Nature 460(7252):259–263

Pang ZP, Yang N, Vierbuchen T, Ostermeier A, Fuentes DR, Yang TQ et al (2011) Induction of human neuronal cells by defined transcription factors. Nature 476(7359):220–223

Pittenger MF, Mackay AM, Beck SC, Jaiswal RK, Douglas R, Mosca JD et al (1999) Multilineage potential of adult human mesenchymal stem cells. Science 284(5411):143–147

Porter DL, Levine BL, Kalos M, Bagg A, June CH (2011) Chimeric antigen receptor-modified T cells in chronic lymphoid leukemia. N Engl J Med 365(8):725–733

Reynolds BA, Weiss S (1992) Generation of neurons and astrocytes from isolated cells of the adult mammalian central nervous system. Science 255(5052):1707–1710

Robinton DA, Daley GQ (2012) The promise of induced pluripotent stem cells in research and therapy. Nature 481(7381):295–305

Rosenberg SA, Yang JC, Sherry RM, Kammula US, Hughes MS, Phan GQ et al (2011) Durable complete responses in heavily pretreated patients with metastatic melanoma using T-cell transfer immunotherapy. Clin Cancer Res 17(13):4550–4557

Rosso F, Marino G, Giordano A, Barbarisi M, Parmeggiani D, Barbarisi A (2005) Smart materials as scaffolds for tissue engineering. J Cell Physiol 203(3):465–470

Sanchez-Ramos J, Cimino C, Avila R, Rowe A, Chen R, Whelan G et al (2012) Pilot study of granulocyte-colony stimulating factor for treatment of Alzheimer's disease. J Alzheimers Dis 31(4):843–855

Sartipy P, Bjorquist P (2011) Concise review: human pluripotent stem cell-based models for cardiac and hepatic toxicity assessment. Stem Cells 29(5):744–748

Seale P, Rudnicki MA (2000) A new look at the origin, function, and "stem-cell" status of muscle satellite cells. Dev Biol 218(2):115–124

Silva EA, Mooney DJ (2007) Spatiotemporal control of vascular endothelial growth factor delivery from injectable hydrogels enhances angiogenesis. J Thromb Haemost 5(3):590–598

Skrtic M, Sriskanthadevan S, Jhas B, Gebbia M, Wang X, Wang Z et al (2011) Inhibition of mitochondrial translation as a therapeutic strategy for human acute myeloid leukemia. Cancer Cell 20(5):674–688

Soen Y, Mori A, Palmer TD, Brown PO (2006) Exploring the regulation of human neural precursor cell differentiation using arrays of signaling microenvironments. Mol Syst Biol 2:37
Stoop R (2008) Smart biomaterials for tissue engineering of cartilage. Injury. 39(1):S77–S87
Takahashi K, Yamanaka S (2006) Induction of pluripotent stem cells from mouse embryonic and adult fibroblast cultures by defined factors. Cell 126(4):663–676
Thomson JA, Itskovitz-Eldor J, Shapiro SS, Waknitz MA, Swiergiel JJ, Marshall VS et al (1998) Embryonic stem cell lines derived from human blastocysts. Science 282(5391):1145–1147
Vierbuchen T, Ostermeier A, Pang ZP, Kokubu Y, Sudhof TC, Wernig M (2010) Direct conversion of fibroblasts to functional neurons by defined factors. Nature 463(7284):1035–1041
Wagers AJ (2012) The stem cell niche in regenerative medicine. Cell Stem Cell 10(4):362–369
Williams D (2005) Environmentally smart polymers. Med Device Technol 16(4):9–10
Wilson JM (2009) Lessons learned from the gene therapy trial for ornithine transcarbamylase deficiency. Mol Genet Metab 96(4):151–157
Zheng Y, Chen J, Craven M, Choi NW, Totorica S, Diaz-Santana A et al (2012) In vitro microvessels for the study of angiogenesis and thrombosis. Proc Natl Acad Sci U S A 109(24):9342–9347
Zweigerdt R (2009) Large scale production of stem cells and their derivatives. Adv Biochem Eng Biotechnol 114:201–235