ORIGINAL ARTICLE

Stem cells: Hype or Miracle Cure?

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ABSTRACTS

Objective: This review aims to evaluate whether stem cell therapy is a miracle cure or hype The analysis will include the proliferation, differentiation, problems, developments and applications of stem cells in a sequential manner.

Study Design: Analytical

Subjects and Methods: This review uses a dialectical approach to objectively assess the therapeutic potential of stem cells. The physico-chemical interactions that underpin the generation, maintenance and differentiation of stem cells are studied. Associated problems and developments in stem cell therapy are compared to understand its current and future applications.

Results and Conclusion: This article suggests that stem cell therapy will be ultimately beneficial. However, it is premature to give a timeline. While it certainly has great potential, the difficulties of practical application must be overcome before it can be called a miracle cure.

Key Words: Mesenchymal Stem cells (MSC). Stem cell therapy.

INTRODUCTION

Stem cell therapy has been idealised as a cure for everything.[1]Many potential beneficiaries have been taken in by this, only to have their hopes dashed later.[2]The hype has made some people sceptical and suspicious of the media.[2]A sizeable population objects to embryo based research on ethical grounds.[3]

Scientists have a cautious approach and focus more on research.[4]The aim of current research is to separate the hype from hope, and define the abilities and limitations of stem cell therapy. Physicians understand that the process of taking cellular therapy from the laboratory to the clinic is long. However, there is hope that stem cell therapy will lead to exciting new possibilities in healthcare.

Stem cells have the definitive properties of self renewal and potency.[5]Self renewal is the ability to divide repeatedly resulting in a clone of cells.[5]Potency is the capacity to differentiate into multiple lineages of more specialised cells.[5]

Stem cells may be totipotent, pleuripotent, multipotent, oligopotent or unipotent each with decreasing differentiation potential.[5]Cells with higher potencies can generate a greater diversity of tissues. More specialised cells have less potency and self renewing ability.

Much research is dedicated to culturing stem cells with the aim of producing large colonies of pleuripotent cell lines. This would ensure a continuous supply of stem cells for further research and therapeutic uses.

METHODOLOGY

Traditionally, stem cells have been cultured on a serum medium containing mouse embryonic fibroblasts (MEFs).[6-7]MEFs provide growth factors that cause the expression of transcription factors, such as Oct-4, Sox-2 and Nanog.[8]These act in concert to promote growth and proliferation of stem cells. Oct-4, Sox-2 and Nanog are markers for pleuripotency.[9-10]

Figure 1*:* The signalling pathways that cause the expression of self renewal genes and the transcription regulatory network.[11]Adapted from (11).

LIF/gp130/JAK/STAT

Leukaemia Inhibitory Factor maintains pleuripotency by inhibiting signals that induce differentiation, accelerating the cell cycle and maintaining the expression of self renewing genes.[12]

WNT signalling

WNT protein regulates the maintenance of embryonic stem cells through the WNT/β-catenin pathway by maintaining pleuripotency factors including Oct-4, ID and STAT3.[12-13]

Transcription Regulatory Network Oct-4 levels must be maintained at a critical range for self renewal.[17]Regulatory regions of Oct-4 and Sox-2 have binding sites, termed HMG/POU

cassettes.[18]These sites are also found on the promoters of target genes and Nanog. Oct-4 and Sox-2 can thus regulate the transcriptional activity of target genes as well as each other.[18]They form a core regulatory network with Nanog.[19]

TGFβ/activin/nodal signalling

Transforming Growth Factor β (TGFβ)/activin/nodal pathway is a critical pathway in the self renewal of human embryonic stem cells.[15]It also blocks differentiation of the neuro-ectoderm line by causing expression of Nanog.[16]

FGF signalling

Fibroblast Growth Factor is necessary to maintain the pleuripotent state. FGF-2 allows formation of compact colonies that are less prone to peripheral differentiation via the Ras-Raf-MAPK signalling cascade/P13K-AKt cell survival pathway.[14]

Feeder cultures potentially expose human cells to chemicals, pathogens and prions making them unsuitable for therapeutic use.[20]Human embryonic stem cells (hESCs) have been grown in a feeder free environment using high concentrations of FGF-2.[21]This also resulted in a twofold increase in hESC expansion compared to an MEF culture.[21]A cocktail of factors including FGF-2, Wnt3A, April, insulin, transferring, albumin and cholesterol has also been used to grow hESCs.[22]

Specialised cell lines are derived from the differentiation of pleuripotent ESCs. In vitro differentiation results in a mixture of different cell lines.[23]Isolating pure cell lines requires the addition of specific growth factors and differentiation inducing agents which cause the transcription of lineage specific genes.[24]Understanding these pathways is crucial to direct the differentiation of particular tissues.

Mesenchymal stem cells are multipotent progenitors found in many tissues such as bone marrow, subcutaneous fat, muscle, periosteum and umbilical cord blood.[25]Their differentiation into cells of mesodermal origin offers a potential cure for many degenerative connective tissue diseases. The advantages (listed below) of MSCs come without the ethical drawbacks associated with ESCs. This has kindled hope of widespread use in the future. Current MSC research is geared towards engineering and transplanting various connective tissues including cartilage, bone and muscle.[26] ***Advantages of MSCs***

1. Widespread location and ability to proliferate extensively (shown to have 40 doublings before self renewal stops) makes them suitable for in vitro expansion and tissue engineering.[27-28]
2. Immune modulatory properties which decrease the probability of graft rejection allowing them to be transplanted easily.[29]
3. Inhibit immune responses against minor histocompatibility antigens such as HY and prevent graft versus host disease when co-transplanted with haematopoietic stem cells.[30]
4. Transfecting MSCs with tumour suppressor genes such as interferon beta has been shown to decrease cancer development in vivo.[30]
5. MSC transplantation is associated with decreased incidence and improved clinical features in autoimmune encephalomyelitis.[31]

Chondrocytes have been generated from bone marrow derived stem cells in cultures, including agarose, alginate, poly (ethylene glycol) and silk.[33]Sox9 transcription factor is the primary mediator in the expression of cartilage specific genes.[34]Cell proliferation increases in hypoxic conditions[35] and with application of shear stresses.[36]These stresses are provided by hydrodynamic bioreactors to increase biochemical content.[37]The cartilage engineered does not hypertrophy and is suitable for therapeutic use.[38]Scaffolds specific for chondrogenesis are being developed.

Neuronal degeneration has long been considered irreversible as highly specialized cells do not divide. Regeneration of neural tissue by stem cells provides hope that Parkinson's disease and demyelinating disorders can be treated in the future. An understanding of neuronal differentiation is necessary to gauge progress in this area.

ESCs enter the neural line as a heterogeneous mixture of cells.[39]To achieve homogeneity a monolayer culture was developed using a defined media.[39]FGF and Notch signalling, along with inhibition of BMP signalling, push the cells into the neural progenitor stage.[40]Addition of FGF8 and Sonic Hedgehog promote differentiation into dopaminergic (DA) neurons.[41]Nurr1, together with other transcription factors expressed by DA neurons, promotes functional maturity.[42]

There is greater DA neuronal differential when stem are grown as spherical neural masses (SNMs). SNMs can be stored for a long time without losing their differentiation capacity.[43]Differentiation into DA neurons only takes 14 days and does not require feeder layers.[43]A high yield (66% purity - highest ever

recorded) of DA neurons is obtained.[43]This is an important milestone for DA neuron generation. It sets the foundation for the application of DA neuron transplantation therapy on a large scale in a clinical setting.

Most of the data on stem cells has come from culturing mESCs. The difference in species raises questions about the validity of the data.[44] Moreover; hESCs divide more slowly than mESCs making their expansion in culture more difficult.[44]Prolonged cultures are required to obtain large colonies of stem cells. This can result in karyotypic abnormalities.[45]Feeder free cultures minimize these risks. However, cultures suitable for therapeutic use are currently some years away.

In vitro differentiation results in a heterogeneous mixture of different cell lines.[46]The concentration of the desired cell line is so small that transplantation is not feasible. More efficient cultures require exact replication of the internal environment. In practice, this is exceedingly difficult and 100% efficiency may never be achieved.

Non-autologous stem cell transplants may result in tissue rejection.[47]Continuous use of immuno-suppressant drugs to counter this will leave the patient susceptible to infections. The immune-regulatory properties of MSCs could provide a solution to this problem.[47]

Transplanted hESC and mESC have been shown to form teratomas in immuno-deficient mice.[48]The link between stem cells and tumour formation is frighteningly strong. Many stem cell markers such Oct-4, Sox-2 and Nanog have been associated with tumourigenesis while c-myc and klf-4 are established proto-oncogenes.[49]The myc family of proto-oncogenes is in fact associated with many tumours and its target genes are co-expressed in both ESC and malignant tumours.[49]

In vitro differentiation aims to regenerate a specific tissue that can then be grafted. This approach can only cure single tissue diseases. Many diseases affect several tissues at once. Generating multiple cell types in vitro has proved to be challenging. Allowing stem cells to differentiate in vivo is also difficult in most cases. Stem cell therapy therefore shows more potential in cases of site specific tissue damage.[50]

Stem cell research is ethically controversial and generates many viewpoints. Social opinion influences laws and state funding policies regarding further research. Much of the controversy revolves around procurement of hESCs leading to ethical arguments similar to the abortion debate.

Similarly, therapeutic cloning is thought by many as devaluing human life and leads to the ethical dilemma of human cloning. There is a consensus against reproductive cloning. However, therapeutic cloning remains ambiguous with different countries adopting different policies towards it.[52]

Stem cell research has recently received an impetus from various innovative techniques. Progress is continuously being made as researchers strive to make stem cell therapies safer and more efficient. These techniques and their limitations are outlined below.

SCNT is a method that produces cloned embryos from which cell lines containing the recipient's DNA can be derived. The nucleus of a somatic cell is extracted and used to displace the nucleus of the donated egg cell.[53]Epigenetic reprogramming back to an undifferentiated state occurs due to chemical factors in the egg cell cytoplasm.[53]The egg is then stimulated to divide until it forms an embryo containing pleuripotent cells that are genetically identical to the donor. These cells can then be cultured and differentiated into various organs and tissues.

The production of induced pleuripotent stem cells was hailed as Sciences 'breakthrough of the year' in 2008.[56]These cells are derived from adult somatic cells through a process of dedifferentiation. This is a reversal of the cellular clock in which a more specialised cell returns to its stem-like nature. In 2006, iPSCs were first produced by transfecting mouse fibroblasts with known stem cell markers. The expression of four key transcription factors, Oct-4, Sox-2, c-myc and Klf4, induced pleuripotency.[57]In 2007, iPSCs were generated from adult human fibroblasts with the transduction of the same four transcription factors.[58]Expression of these transcription factors at an optimal ratio of 3:1:1:1 increases the efficiency ofproducing iPSCs.[59]Other combinations of transcription factors (Oct-4, Sox-2, Nanog and Lin28) have also been transduced to generate iPSCs.[60]These cells express similar markers to hESCs and have been differentiated into functional neurons[61], glial cells[61]and cardiomyocytes.[62-63]

Stem cell research has become increasingly dependent on biotechnology. DNA chip technology allows researchers to simultaneously analyse thousands of genes.[67]Machine vision technology generates images of cells and automates culturing,

monitoring and analysis of embryonic cells.[68]Tissue engineering has advanced with the production of improved scaffolds which support three dimensional tissue production.[69]Establishing bioreactors capable of supporting efficient proliferation and differentiation will be a massive step for the large scale application of cellular therapy.

DISCUSSION

The many potential and practical applications of stem cell therapy are discussed below in the light of some common diseases to better evaluate its future role.

Haematopoietic stem cell transplantation (HSCT) has been used for years to treat leukaemias and lymphomas. Both malignant and normal cells are destroyed by chemotherapy and replaced with transplanted stem cells that differentiate into functional cells in vivo.[70]Traditionally the bone marrow is used as a source of stem cells. The use of peripheral blood is increasing as it provides a bigger graft.[71]There is interest in storing and utilising umbilical cord blood since there is decreased risk of graft versus host disease but the quantities obtained are small.[72]It is a potential therapy for type 1 DM and cardiovascular disease with encouraging results in clinical trials and animal models.[73]

In vitro production of dopaminergic (DA) neurons can replace the dead neurons in the midbrain. Transplanted DA neurons have shown statistically significant loco-motor functional recovery in hemi-parkinsonian rats.[74]Motor improvement was also seen after transplantation of mesencephalic progenitors (CSM14.1 cells).[75]

Demyelination is the primary pathology in multiple sclerosis, leukodystrophies and spinal cord injuries. Oligodendrocytes can be differentiated in culture from pleuripotent stem cells[76]and forebrain sub-ventricular zone progenitor cells can differentiate into astrocytes[77] upon transplantation. Transplantation of these cells has promoted functional recovery in rats.[78-79]Transplanting neural stem cells transduced with the Olig2 transcription factor enhances myelination in the white matter and improves locomotion in rats.[80]However, cellular therapy has limited potential in multiple sclerosis because axon regeneration is required at several sites. Disorders with localized myelin sheath damage such as optic neuritis will be easier to treat.

Osteoarthritis affects an estimated 8.5 million people in the UK.[81]The pathology involves articular cartilage destruction. MSCs can migrate to injured sites and undergo site specific differentiation to regenerate articular cartilage.[82]Their immune modulatory properties also reduce inflammation and ease pain in immune mediated diseases like rheumatoid arthritis. MSC transplantation has been shown to stimulate regeneration of the medial meniscus and decrease joint degeneration in a goat.[82]Osteochondral progenitor cells have been used to repair full-thickness defects in the articular cartilage in the knees of rabbits.[83]In vitro cartilage engineering is also a potential therapeutic approach in which cartilage tissue is generated and engrafted into the joint cavity.[84]

Type 1 diabetes is an insulin deficiency resulting from autoimmune mediated destruction of insulin-producing β-cells in the pancreatic islets. Immunosuppression with HSCT has been shown to improve clinical features of early type 1 DM in humans. 23 patients became insulin independent for more than a month.[85]Human cord blood stem cells differentiate into islets when transplanted in Type 1 DM mice and improve hyperglycaemia in obesity induced diabetic mice.[86]Splenic mesenchymal cells have also been shown to differentiate into β-cells in rodents and reverse diabetes.[87]They can also keep islet destruction in check due to their immune regulatory properties.

CONCLUSION

Applications in regenerative medicine require the development of safer and more efficient cultures, increased use of biotechnology, more clinical trials and increased investment. Above all, it requires time, so one should not expect immediate benefits. However, stem cells do have immediate uses in drug discovery, toxicology, functional genomics and basic cancer research so they may still contribute to cures in an indirect manner. Stem cell research has followed a dialectical paradigm. New developments have been met with new problems. Whether stem cell therapy is a miracle cure or not is debatable. However, it is certainly not hype.

REFERENCES

1. Rosenthal N. Youthful prospects for human stem-cell therapy. In another few decades, revised attitudes toward stem cells could lead to disease prevention and life extension. *EMBO Rep* 2005; 6:530-4.

2. V.L.Peddie, Porter M, Counsell C, Caie L, Pearson D, Bhattacharya S. 'Not taken in by media hype': how potential donors, recipients and members of the general public perceive stem cell research.*Human Reproduction* 2009; 24(5):1106-13.
3. Nisbet MC. Public Opinion about Stem Cell Research and Human Cloning *Public Opinion Quarterly* 2004; 68(1):131-54.
4. Wainwright SP, Williams C, Michael M, Farsides B, Cribb A. From bench to bedside? Biomedical scientists' expectations of stem cell science as a future therapy for diabetes *Social Science & Medicine*2006; 63(8):2052-64.
5. Smith A. A glossary for stem-cell biology. *Nature* 2006; 441:1060.
6. McDevitt TC, Palecek SP. Innovation in the culture and derivation of pluripotent human stem cells. *Curr Opin Biotechnol* 2008; 19(5):527-33.
7. Park JH, Kim SJ, Oh EJ, Moon SY, Roh SI, Kim CG, et al. Establishment and maintenance of human embryonic stem cells on STO, a permanently growing cell line. *Biol Reprod* 2003; 69(6):2007-14.
8. Tsuji Y, Yoshimura N, Aoki H, Sharov AA, Ko MS, Motohashi T, et al. Maintenance of undifferentiated mouse embryonic stem cells in suspension by the serum- and feeder-free defined culture condition. *Dev Dyn* 2008; 237(8):2129-38.
9. Cai J, Chen J, Liu Y, Miura T, Luo Y, Loring JF, et al. Assessing self-renewal and differentiation in hESC lines. *Stem Cells* 2006; 24(3):516-30.
10. Chambers I, Tomlinson SR. The transcriptional foundation of pluripotency. *Development* 2009; 136(14):2311-22.
11. SABiosciences. Nanog in Mammalian ESC Pluripotency. Available from:http://www.sabiosciences.com/pathway.php?sn=Nanog_in_Mammalian_ESC_Pluripotency [Accessed 20/03/2010]
12. YuXiao L, Lei J, Yue T, YunFang W, XueTao P. The molecular mechanism of embryonic stem cell pluripotency and self-renewal. *Sci China Ser C-Life Sci* 2007; 50(5):619-23.
13. Lensch MW, Daheron L, Schlaeger TM. Pluripotent Stem Cells and Their Niches. *Stem cell reviews and reports* 2007; 2(3):185-201.
14. Eiselleova L, Matulka K, Kriz V, Kunova M, Schmidtova Z, Neradil J, et al. A complex role for FGF-2 in self-renewal, survival, and adhesion of human embryonic stem cells. *Stem Cells* 2009; 27(8):1847-57.
15. James D, Levine AJ, Besser D, Hemmati-Brivanlou A. TGFβ/activin/nodal signaling is necessary for the maintenance of pluripotency in human embryonic stem cells *Dev Bio* 2005;132:1273-82.
16. Vallier L, Mendjan S, Brown S, Chng Z, Teo A, Smithers LE, et al. Activin/Nodal signalling maintains pluripotency by controlling Nanog expression. *Development* 2009; 136(8):1339-49
17. Niwa H, Miyazaki J, Smith AG. Quantitative expression of Oct-3/4 defines differentiation, dedifferentiation or self-renewal of ES cells. *Nat Genet* 2000; 24(4):372-6.
18. Rizzino A. Sox2 and Oct-3/4: A Versatile Pair of Master Regulators that Orchestrate the Self-renewal and Pluripotency of Embryonic Stem Cells by Functioning as Molecular Rheostats. *Wiley Interdiscip Rev Syst Biol Med* 2009; 1(2):228-36.
19. Boyer LA, Lee TI, Cole MF, Johnstone SE, Levine SS, Zucker JP, et al. Core Transcriptional Regulatory Circuitry in Human Embryonic Stem Cells. *Cell* 2005; 122:947-56.
20. Vallier L, Pedersen R. Differentiation of human embryonic stem cells in adherent and in chemically defined culture conditions. *Curr Protoc Stem Cell Biol* 2008; Chapter 1: Unit 1D 4 1-1D 4 7
21. Li Y, Powell S, Brunette E, Lebkowski J, Mandalam R. Expansion of human embryonic stem cells in defined serum-free medium devoid of animal-derived products. *Biotechnol Bioeng* 2005; 91(6):688-98.
22. Lu J, Hou R, Booth CJ, Yang SH, Snyder M. Defined culture conditions of human embryonic stem cells. *Proc Natl Acad Sci U S A* 2006; 103(15):5688-93
23. Bratt-Leal AM, Carpenedo RL, McDevitt TC. Engineering the embryoid body microenvironment to direct embryonic stem cell differentiation. *Biotechnol Prog* 2009; 25(1):43-51
24. Levenberg S, Huang NF, Lavik E, Rogers AB, Itskovitz-Eldor J, Langer R. Differentiation of human embryonic stem cells on three-dimensional polymer scaffolds. *Proc Natl Acad Sci U S A* 2003; 100(22):12741-6
25. Chen FH, Tuan RS. Mesenchymal stem cells in arthritic diseases. *Arthritis Res Ther* 2008; 10(5):223.

26. Tuan RS, Boland G, Tuli R. Adult mesenchymal stem cells and cell-based tissue engineering.*Arthritis Res Ther* 2003; 5(1):32-45.
27. Javazon EH, Beggs KJ, Flake AW. Mesenchymal stem cells: paradoxes of passaging. *Exp Hematol*2004; 32(5):414-25
28. Wu W, Zhou Y, Tan W. [In vitro culture of bone marrow-derived mesenchymal stem cells in a chemically-defined serum-free medium]. *Sheng Wu Gong Cheng Xue Bao* 2009; 25(1):121-8.
29. Haniffa MA, Collin MP, Buckley CD, Dazzi F. Mesenchymal stem cells: the fibroblasts' new clothes? *Haematologica* 2009; 94(2):258-63.
30. Krampera M, Franchini M, Pizzolo G, Aprili2 G. Mesenchymal stem cells: from biology to clinical use *Blood Transfus* 2007;5(3):120-29.
31. Zappia E, Casazza S, Pedemonte E, Benvenuto F, Bonanni I, Gerdoni E, et al. Mesenchymal stem cells ameliorate experimental autoimmune encephalomyelitis inducing T-cell anergy. *Blood* 2005; 106(5):1755-61.
32. Tuan RS, Boland G, Tuli R. Adult mesenchymal stem cells and cell-based tissue engineering.*Arthritis Res Ther* 2003; 5(1):32-45.
33. Chung C, Burdick JA. Engineering cartilage tissue. *Adv Drug Deliv Rev* 2008; 60(2):243-62.
34. Hardingham TE, Oldershaw RA, Tew SR. Cartilage, SOX9 and Notch signals in chondrogenesis. *J Anat* 2006; 209(4):469-80.
35. Ma T, Grayson WL, Frohlich M, Vunjak-Novakovic G. Hypoxia and stem cell-based engineering of mesenchymal tissues. *Biotechnol Prog* 2009; 25(1):32-42.
36. Zuscik MJ, Hilton MJ, Zhang X, Chen D, O'Keefe RJ. Regulation of chondrogenesis and chondrocyte differentiation by stress. *J Clin Invest* 2008; 118(2):429-38.
37. Hwang NS, Varghese S, Elisseeff J. Controlled differentiation of stem cells. *Adv Drug Deliv Rev*2008; 60(2):199-214.
38. Hwang NS, Varghese S, Elisseeff J. Derivation of chondrogenically-committed cells from human embryonic cells for cartilage tissue regeneration. *PLoS One* 2008; 3(6):e2498.
39. Abranches E, Silva M, Pradier L, Schulz H, Hummel O, Henrique D, et al. Neural differentiation of embryonic stem cells in vitro: a road map to neurogenesis in the embryo. *PLoS One* 2009; 4(7):e6286.
40. Dhara SK, Stice SL. Neural differentiation of human embryonic stem cells. *J Cell Biochem* 2008; 105(3):633-40
41. Yan Y, Yang D, Zarnowska ED, Du Z, Werbel B, Valliere C, et al. Directed differentiation of dopaminergic neuronal subtypes from human embryonic stem cells. *Stem Cells* 2005; 23(6):781-90.
42. Sacchetti P, Carpentier R, Segard P, Olive-Cren C, Lefebvre P. Multiple signaling pathways regulate the transcriptional activity of the orphan nuclear receptor NURR1. *Nucleic Acids Res* 2006; 34(19):5515-27.
43. Cho MS, Lee YE, Kim JY, Chung S, Cho YH, Kim DS, et al. Highly efficient and large-scale generation of functional dopamine neurons from human embryonic stem cells. *Proc Natl Acad Sci U S a*2008; 105(9):3392-7.
44. Paul G, Li JY, Brundin P. Stem cells: hype or hope? *Drug Discov Today* 2002; 7(5):295-302.
45. Sareen D, McMillan E, Ebert AD, Shelley BC, Johnson JA, Meisner LF, et al. Chromosome 7 and 19 trisomy in cultured human neural progenitor cells. *PLoS One* 2009; 4(10):e7630.
46. Yao S, Chen S, Clark J, Hao E, Beattie GM, Hayek A, et al. Long-term self-renewal and directed differentiation of human embryonic stem cells in chemically defined conditions. *Proc Natl Acad Sci U S A*2006; 103(18):6907-12.
47. Batten P, Rosenthal NA, Yacoub MH. Immune response to stem cells and strategies to induce tolerance. *Philos Trans R Soc Lond B Biol Sci* 2007; 362(1484):1343-56.
48. Dressel R, Schindehütte J, Kuhlmann T, Elsner L, Novota P, Baier PC, et al. The Tumorigenicity of Mouse Embryonic Stem Cells and In Vitro Differentiated Neuronal Cells Is Controlled by the Recipients' Immune Response. *PLoS ONE* 2008; 3(7):e2622.
49. Knoepfler PS. Deconstructing stem cell tumorigenicity: a roadmap to safe regenerative medicine.*Stem Cells* 2009; 27(5):1050-6.
50. Mazzocchi F. Complexity in biology. Exceeding the limits of reductionism and determinism using complexity theory. *EMBO Rep* 2008; 9(1):10-14.
51. StemGen. Stem cell policy http://www.stemgen.org/mapworld.cfm [Accessed 21/03/2010]

52. Mayor S. UK body calls on UN to allow therapeutic cloning. *BMJ* 2004; 329(7472):938
53. Tian XC, Kubota C, Enright B, Yang X. Cloning animals by somatic cell nuclear transfer--biological factors. *Reprod Biol Endocrinol* 2003; 1:98.
54. Kfoury C. Therapeutic cloning: promises and issues. *Mcgill J Med* 2007; 10(2):112-20.
55. Hipp J, Atala A. Tissue engineering, stem cells, cloning, and parthenogenesis: new paradigms for therapy. *J Exp Clin Assist Reprod* 2004; 1(1):3.
56. Vogel G. Breakthrough of the Year: Reprogramming cells. *Science* 2008; 322(5909):1766-67
57. Takahashi K, Yamanaka S. Induction of pluripotent stem cells from mouse embryonic and adult fibroblast cultures by defined factors. *Cell* 2006; 126(4):663-76
58. Takahashi K, Tanabe K, Ohnuki M, Narita M, Ichisaka T, Tomoda K, et al. Induction of pluripotent stem cells from adult human fibroblasts by defined factors. *Cell* 2007; 131(5):861-72.
59. Papapetrou EP, Tomishima MJ, Chambers SM, Mica Y, Reed E, Menon J, et al. Stoichiometric and temporal requirements of Oct4, Sox2, Klf4, and c-Myc expression for efficient human iPSC induction and differentiation. *Proc Natl Acad Sci U S A* 2009; 106(31):12759-64
60. Yu J, Vodyanik MA, Smuga-Otto K, Antosiewicz-Bourget J, Frane JL, Tian S, et al. Induced pluripotent stem cell lines derived from human somatic cells. *Science* 2007; 318(5858):1917-20.
61. Hu BY, Weick JP, Yu J, Ma LX, Zhang XQ, Thomson JA, et al. Neural differentiation of human induced pluripotent stem cells follows developmental principles but with variable potency. *Proc Natl Acad Sci U S A* 2010; 107(9):4335-40.
62. Gai H, Leung EL, Costantino PD, Aguila JR, Nguyen DM, Fink LM, et al. Generation and characterization of functional cardiomyocytes using induced pluripotent stem cells derived from human fibroblasts. *Cell Biol Int* 2009; 33(11):1184-93.
63. Zhang J, Wilson GF, Soerens AG, Koonce CH, Yu J, Palecek SP, et al. Functional cardiomyocytes derived from human induced pluripotent stem cells. *Circ Res* 2009; 104(4):e30-41.
64. Stadtfeld M, Nagaya M, Utikal J, Weir G, Hochedlinger K. Induced Pluripotent Stem Cells Generated Without Viral Integration. *Science*; 322(5903).
65. Nakagawa M, Koyanagi M, Tanabe K, Takahashi K, Ichisaka T, Aoi T, et al. Generation of induced pluripotent stem cells without Myc from mouse and human fibroblasts. *Nature Biotech* 2007; 26:101-06.
66. Zhou H, Wu S, Joo JY, Zhu S, Han DW, Lin T, et al. Generation of Induced Pluripotent Stem Cells Using Recombinant Proteins. *Cell Stem Cell* 2009; 4(5):381-84.
67. Lanza R, Gearhart J, Hogan B, Melton D, Pederson R, Thomas ED, et al. *Essentials of Stem Cell Biology*. pp xvii.San Diego: Academic Press, 2005.
68. Narkilahti S, Rajala K, Pihlajamaki H, Suuronen R, Hovatta O, Skottman H. Monitoring and analysis of dynamic growth of human embryonic stem cells: comparison of automated instrumentation and conventional culturing methods. *Biomed Eng Online* 2007; 6:11.
69. Placzek MR, Chung I-M, Macedo HM, Ismail S, Blanco TM, Lim M, et al. Stem cell bioprocessing: fundamentals and principles. *J R Soc Interface* 2009; 6(32):209–32.
70. Duran-Struuck R, Dysko RC. Principles of bone marrow transplantation (BMT): providing optimal veterinary and husbandry care to irradiated mice in BMT studies. *J Am Assoc Lab Anim Sci*2009; 48(1):11-22.
71. Cutler C, Antin JH. Peripheral blood stem cells for allogeneic transplantation: a review. *Stem Cells*2001; 19(2):108-17.
72. Gluckman E, Rocha V. Cord blood transplantation: state of the art. *Haematologica* 2009; 94(4):451-4.
73. Brown JA, Boussiotis VA. Umbilical cord blood transplantation: basic biology and clinical challenges to immune reconstitution. *Clin Immunol* 2008; 127(3):286-97.
74. Yang D, Zhang ZJ, Oldenburg M, Ayala M, Zhang SC. Human embryonic stem cell-derived dopaminergic neurons reverse functional deficit in parkinsonian rats. *Stem Cells* 2008; 26(1):55-63.
75. Haas SJ-P, Petrov S, Kronenberg G, Schmitt O, Wree A. Orthotopic transplantation of immortalized mesencephalic progenitors (CSM14.1 cells) into the substantia nigra of hemiparkinsonian rats induces neuronal

differentiation and motoric improvement. *J Anat* 2008; 212(1):19-30.
76. Hu BY, Du ZW, Zhang SC. Differentiation of human oligodendrocytes from pluripotent stem cells. *Nat Protoc* 2009; 4(11):1614-22.
77. Milosevic A, Noctor SC, Martinez-Cerdeno V, Kriegstein AR, Goldman JE. Progenitors from the postnatal forebrain subventricular zone differentiate into cerebellar-like interneurons and cerebellar-specific astrocytes upon transplantation. *Mol Cell Neurosci* 2009; 39(3):324-34.
78. Davies JE, Huang C, Proschel C, Noble M, Mayer-Proschel M, Davies SJ. Astrocytes derived from glial-restricted precursors promote spinal cord repair. *J Biol* 2006; 5(3):7.
79. Bambakidis NC, Miller RH. Transplantation of oligodendrocyte precursors and sonic hedgehog results in improved function and white matter sparing in the spinal cords of adult rats after contusion.*Spine J* 2004; 4(1):16-26.
80. Hwang DH, Kim BG, Kim EJ, Lee SI, Joo IS, Suh-Kim H, et al. Transplantation of human neural stem cells transduced with Olig2 transcription factor improves locomotor recovery and enhances myelination in the white matter of rat spinal cord following contusive injury. *BMC Neurosci* 2009; 10:117
81. NHS. Osteoarthritis: Available from: http://www.nhs.uk/Conditions/Osteoarthritis/Pages/Introduction.aspx [Accessed 23/03/2010]
82. Murphy JM, Fink DJ, Hunziker EB, Barry FP. Stem cell therapy in a caprine model of osteoarthritis. *Arthritis Rheum* 2003; 48(12):3464-74.
83. Couri CE, Voltarelli JC. Stem cell therapy for type 1 diabetes mellitus: a review of recent clinical trials. *Diabetol Metab Syndr* 2009; 1(1):19.
84. Wakitani S, Goto T, Pineda SJ, Young RG, Mansour JM, Caplan AI, et al. Mesenchymal cell-based repair of large, full-thickness defects of articular cartilage. *J Bone Joint Surg Am* 1994; 76(4):579-92.
85. Naujoks C, Meyer U, Wiesmann HP, Jasche-Meyer J, Hohoff A, Depprich R, et al. Principles of cartilage tissue engineering in TMJ reconstruction. *Head Face Med* 2008; 4:3.
86. Voltarelli JC, Couri CEB, Stracieri ABPL, Oliveira MC, Moraes DA, Pieroni F, et al. Autologous Nonmyeloablative Hematopoietic Stem Cell Transplantation in Newly Diagnosed Type 1 Diabetes Mellitus. *Jama* 2007; 297:1568-76.
87. Couri CE, Voltarelli JC. Stem cell therapy for type 1 diabetes mellitus: a review of recent clinical trials. *Diabetol Metab Syndr* 2009; 1(1):19.
88. Lee DD, Grossman E, Chong AS. Cellular therapies for type 1 diabetes. *Horm Metab Res* 2008; 40(2):147-54