REVIEW

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Stem cells and regenerative medicine: potentials and realities for rhinology*

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SUMMARY

It is widely believed that regenerative medicine, including stem cell-based technologies, will revolutionise healthcare in decades to come. Stem-cell treatments are already a reality and tissue engineering is moving deeper and deeper into the clinic. Various forms of stem cell and scaffold are in clinical trials and can be used alone, in combinations or supported by conventional treatments, such as drugs and free tissue transfer. It is likely that rhinology will also feel the winds of change very shortly. We review the present state-of-the art and a view of the future potential for regenerative medicine to influence care of patients with rhinologic disorders.

Key words: stem cells, regenerative medicine, rhinology

INTRODUCTION

Regenerative medicine is the restoration of form or function by regeneration or repair of tissues or organs. Its repertoire includes stem cells, tissue engineering, biomaterials, gene therapy and personalised medicine. Conventionally, replacement and repair is either by using artificial implants or by transplantation of tissues [(1)]. Such interventions have been hindered by factors such as immune rejection, limited supply and donor site morbidity. Both disease and therapy in rhinology can result in structural and functional defects. Reconstructive surgery has often required autologous cartilage grafting in order to correct skeletal defects. Trauma and disease of the musculoskeletal system results in considerable morbidity, which has also previously been addressed by means of synthetic prosthetic implant devices. All of these strategies are failing to deal with the increasing demand for treatments for this spectrum of clinical problems. There are many reasons for this, including the shortage of suitable graft material, problems in harvesting sufficient material to fill large defects adequately, implant biocompatibility and implant failure. Alternative approaches are needed therefore to address this growing problem [(2)]. To ensure consistency in this rapidly evolving field, all terms used in this review are based upon the glossary defined by Mason et al. in Regenerative Medicine [(3)].

THE GROWING CHALLENGE

One of the chief goals of medicine has always been to overcome the debilitating effects of organ and tissue loss. For many centuries, removal of the diseased tissue was the only option. Greater understanding of how organs function led to the realization that, in some situations, a synthetic replacement might be used to treat disease. Other advances in areas such as antiseptics, antibiotics and improved hygiene have all contributed to a dramatic increase in human longevity, leading to a greater need for replacement of tissues. Limb prosthetics was the first area to make use of synthetic materials in a way, which substantially advanced patient care [(4)]. Since then, medical implants have radiated into a staggering variety of numbers and designs [(5)]. Millions of patients have had their quality of life markedly enhanced by the development and deployment in the clinical setting of implants such as total joint prostheses, cardiovascular stents and artificial heart valves.

The socio-economic costs of treating tissue loss and organ failure in an increasingly ageing population (predominantly in the Western world) are vast [(6)] and, while artificial implants have significantly improved the quality of life for many patients, so far it has not been possible to overcome their major failing, namely limited lifespan. Implants that do not integrate with the host tissue are subject to wear and thus eventually fail, implant survivability usually being no greater than 15 years [(7)]. The basis of this problem lies in the selection of materials for use in implants. Implants constructed from bio-inert materials have proved to be mechanically durable but often unsatisfactory in terms of their biocompatibility, sometimes causing inflammatory responses [(8)]. Improving biocompatibility has therefore been perhaps the biggest driver of innovation in medical device design in recent years. The number of "ideal" new materials being proposed for medical devices and their complexity is continuing to grow each year, as researchers around the world continue their quest for improvement [(9)].

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However, these modifications can lead to a compromise on the engineering aspects of the implant to improve its biocompatibility.

Possibly the overarching challenge now facing biomedical science is one of its own making - how to address the progressive increase in patient longevity. The effect of increased patient longevity is twofold – many more patients now require these treatments and the treatments themselves are required to have longer lifetimes as well. A shift in emphasis is therefore needed, from the current methods for the replacement of tissues to more biological approaches including the regeneration of tissues [10]. One of the fundamental properties of living tissue in a multi-cellular organism is its capacity to adapt and remodel to physiological and environmental cues. One of the shortcomings observed in the use of synthetic implants is the inability to perceive the local conditions and respond in an appropriate fashion, which can be a major factor in implant failure. The next step in developing clinical implants is therefore to use more biological, bioactive materials, ones that provide the appropriate biological signals, which can either elicit a regenerative response at the site of damage *in vivo* or be used to grow tissue *in vitro* for subsequent implantation. In order to achieve this, materials must have sophisticated properties that go beyond the basics of enhancing cell adhesion and minimizing inflammatory responses. Combining these materials with cells will allow the fabrication of living tissue implants which have full biological function, the ability to respond to environmental changes and which possess a significantly longer lifespan than current implant devices. In this stem cells are emerging as important part of the cell armory.

REGENERATIVE MEDICINE

Regenerative medicine is a multidisciplinary area of research aimed at regeneration of tissues and restoration of function of organs through implantation of cells/tissues grown outside the body or stimulating cells to grow into an implanted matrix [11]. The general principle of tissue engineering involves combining living cells with a natural or synthetic support or scaffold to produce a three-dimensional living tissue construct that is functionally, structurally and mechanically equivalent to the tissue it has been designed to replace [11]. A major advantage of this approach is that tissues can be designed to grow in such a way that they more precisely match the requirements of the individual in terms of size, shape and immunological compatibility, minimising the need for further treatment. The steps involved in the engineering of tissues and organs are cell harvest from the donor site, seeding of cells onto a scaffold, stimulation of cellular proliferation, maintaining or stimulating cellular differentiation and, finally, transplant of the living tissue or organ to the patient.

For all regenerative medicine strategies that involve the engineering of tissues *ex vivo* or cell therapy to augment endogenous regeneration, we must consider the source of cells that can potentially be used in human patients. Current options include: autologous, tissue-specific stem/progenitor cells isolated from peripheral blood and various tissues of the body; allogeneic, xenogeneic (generally encapsulated) or autologous cells derived from bone marrow; pluripotent human embryonic stem (hES) cells; and induced pluripotent stem (iPS) cells.

Tissue-derived stem/progenitor cells

Although autologous tissue-specific stem and progenitor cells (i.e. skeletal muscle, cardiac, neural, skin, etc) would be most ideal for clinical application due to lack of immune rejection issues, these cells are usually rare and difficult to propagate *ex vivo* in numbers large enough for therapeutic applications. An exception may be the so-called endothelial progenitor cells that have been derived from multiple adult human tissues, including blood [12], which is easily accessible for autologous therapies. However, the exact phenotype of these cells is controversial, and they may represent multiple different types of cells with distinct cellular origins and potential [13].

Thus, we must consider other possible sources such as bone marrow, which contains not only hematopoietic stem and progenitor cells that have been previously used to treat hematopoietic disorders, but also mesenchymal stem cells (MSCs), which exhibit multi (if more limited) lineage potential. MSC's actually occur throughout the body, and are now thought be synonymous with pericytes [14], a previously enigmatic cell type found adjacent to blood vessels of all types (and therefore particularly prevalent in bone marrow and fat). MSC's also have important immunoregulatory activity, particularly in the local reduction of pro-inflammatory stimuli at sites of injury [15]. Indeed, this 'anti-inflammatory' effect forms the basis of a number of ongoing clinical trials presently for diseases ranging from graft-versus-host disease to rheumatoid arthritis [16]. Whilst bone marrow and fat are the most well tried sources of MSC's, new sources continue to open up, such as amniotic fluid derived stem cells [17] and the recent discovery of stem cells in urine (possibly exfoliated pericytes) [18]. The true potential of these exciting sources is as yet unknown.

Human embryonic stem cells

The use of human ES cells has been investigated as an alternative to stem and progenitor cells derived from adult tissues. Human ES cells were first isolated by James Thomson from the inner cell mass of human blastocysts [19], and provide a potentially unlimited supply of all cell types of the body. Researchers have taken advantage of pre-implantation genetic diagnosis and have utilized embryos containing genetic disorders to generate disease-specific human ES cell lines [20] to study the underlying mechanism(s) of disease progression. However, for the clinical potential of hES cells to be realized, a number of obstacles must be overcome, including the need for: improved culture conditions for hES cells to allow large-scale expansion under GMP conditions; efficient differentiation of hES cells to specific lineages *in vitro*; integration of transplanted cells in a physiologically useful form; and, critical-

ly, prevention of immune rejection. Since one of the main goals of regenerative medicine is to reduce the need for conventional transplantation with all its side-effects and other issues, the latter is an important consideration. However, this may not be insurmountable, particularly when applied to 'immunologically privileged sites' such as the CNS, eye and joint spaces. Nevertheless, ethical and regulatory issues to one side, much work is needed to optimize their clinical use.

Human induced pluripotent stem cells

Thus, the stem cell field pushed to identify a non-embryonic source of pluripotent stem cells. In 2007, a major breakthrough occurred with the generation of human induced pluripotent stem cells [21]. This was achieved by retrovirally transducing somatic cells (fibroblasts) with transcription regulators associated with hES cell pluripotency, Oct3/4, Klf4, Sox2 and c-myc. The resulting cells were similar to hES cells in their phenotype, promoter methylation status, and ability to give rise to all three germ layers *in vivo*. The drawbacks of this initial method of "reprogramming" somatic cells were the utilization of oncogenes and viral transduction, both of which pose health issues for human subjects. Since the initial derivation of iPS cells, there have been many reports demonstrating successful reprogramming without the use of oncogenes [22] and using alternate delivery methods for reprogramming factors such as the transposon system and episomal factors [23,24]. In addition, while fibroblasts were the first cell type reprogrammed, there have since been other cell populations to be successfully reprogrammed such as hair keratinocytes and blood cells [25,26], demonstrating that this reprogramming ability is not unique to fibroblasts.

While human iPS cell derivation has revolutionized the pluripotent stem cell field, their differentiation potential has to be fully tested and compared to hES cells, to ensure that they could be a suitable equivalent. Thus far, human iPS cells have been reported to give rise to many cell types including adipocytes, cardiomyocytes, endothelial cells, hematopoietic cells, neural cells and pancreatic insulin-producing cells [27-31]. However, there is significant variability among human iPS cell lines in their ability to differentiate into various lineages, which may be dependent upon their cell type of origin and reprogramming strategy. Thus, many additional studies are needed to determine whether human iPS cells can serve as a suitable alternative to hES cells for the generation of all cell types for clinical applications (for review see Vats et al., 2005) [32].

OLFACTION AND OLFACTORY ENSHEATHING CELLS

There are many potential therapeutic use of cells engineered to support regeneration. Of special interest to rhinologists are olfactory ensheathing cells. These are a class of glial cells that support regeneration of olfactory receptor neurons in the olfactory epithelium. When transplanted to other regions of the brain they may support regeneration of other classes of neurons [33]. They also support axon regeneration and have been successfully employed in rat models of spinal cord regeneration [34,35]. Periosteal stem cells and muscle stem cells have been engineered to express BMP, Shh or VEGF and then used in animal models of bone repair [36,37]. Expression of the growth factors improved the quality of repair. The mammalian olfactory system has the distinction of being the only part of the central nervous system undergoing continuous renewal. Olfactory receptor neurons have half-lives of a few weeks and are renewed by division of stem cells in the olfactory epithelium which give rise both to neurons and to supporting cells [38,39]. Their number is self-regulated by secretion of inhibitory factors including GDF-11 [40], and FGFs and BMPs [41] from the olfactory neurons. Newly generated receptor neurons extend axons through the cribriform plate to accurately reinnervate particular olfactory glomeruli specific for each odour receptor type (c. 400 in humans, c. 900 in rodents). Their regeneration is promoted by growth factors secreted by olfactory ensheathing cells (OEC) located beneath the olfactory epithelium [33]. OEC are a form of glia, and there is much interest in their possible therapeutic use to stimulate axon regeneration in other regions of the brain [33-35,42]. Not only are olfactory receptor neurons continuously renewed, but also their target neurons in the olfactory bulb. These form from neural stem cells in the sub-ventricular zones of the lateral ventricles [43], and then migrate as a chain of neural precursor cells [44,45] to form neurons in the olfactory bulb.

Nasal Cartilage

Although articular cartilage has a low capacity for repair, mesenchymal stem cells with the capacity to form cartilage, bone or adipose tissue can be extracted from it [46]. Autologous transplantation of amplified stem cells to repair damaged cartilage is reported to have good outcomes [47,48] which may in future be enhanced by engineering them to express growth factors [36], and by transplanting them embedded in a matrix rather than in suspension [49,50]. Human nasal septum is a good source of chondrogenic cells with the potential to be used to engineer transplants for restorative surgery in otolaryngology [51]. Furthermore, for reasons which remain unclear, cranial and bronchial cartilage has the capacity to heal, a phenomenon absent, but highly desirable, in adult articular cartilage. Current treatment methods have focussed on autologous cell transplantation (MSC or chondrocytes) with or without supporting scaffolds. Autologous chondrocyte transplantation was first used in humans in 1987 and showed stable long term results in patients with single condyle lesions particularly. It was felt that a better understanding of the repair mechanism induced by cultured chondrocytes and the regulatory mechanisms controlling differentiation would be of benefit in developing new cartilage treatments [47,52] introduced a new cell technology, in which cultured chondrocytes were transplanted into defects, raising the expectation of repairing damaged articular cartilage and highlighting the importance of the micro-environment.

Concerns over the maintenance of the chondrocyte phenotype in monolayer culture *in situ*, the leaking of chondrocytes from the primary site and uneven distribution in the three dimensional space were raised and have led to alternative methods of cartilage regeneration being investigated.

In 2008, MSC-derived chondrocytes were used successfully to seed the external surface decellularized human donor trachea which formed the world's first stem cell based organ transplant in a 30 year old woman with end-stage tracheobronchial stenosis [53]. This important first step in the clinical application of stem cells for airway reconstruction shows clearly what should be possible for the generation of nasal cartilage, for example in patients with rhinectomy, large septal defects or, theoretically, for cosmetic purposes.

Differentiation of embryonic stem (ES) cells has been directed towards specific phenotypes, including chondrocytes, by modification of culture conditions, *in vitro*, previously. Co-culture systems have been shown to drive and promote the differentiation of cells towards the mature phenotype, highlighting the potential influence of the micro-environment. Chondrogenic differentiation has now been demonstrated *in vitro* and *in vivo* with human ES cells [54]. A deeper understanding of the mechanisms of stem cell activation may generate technologies for controlled regeneration of particular tissues, using their intrinsic stem cell populations. The demonstration that bone marrow MSC can be activated to differentiate into specific cell types and to home to particular tissues offers the possibility of stimulating tissue regeneration by intravenous infusion of appropriate cell populations. Cultured stem cells may be surgically grafted into sites where regeneration is required. One can only speculate on the speed with which these speculations can become clinical reality.

Nasal epithelium

Nasal epithelial tissue was used as a source of respiratory epithelial cells to seed a human tracheal graft [53]. Although cultured bronchial cells were ultimately employed, the nasal cells proliferated well for several weeks, suggesting the existence of progenitor or 'stem-cell like' cells in this tissue. Such cells are known to exist in the trachea and bronchi [55], and if their presence in the nose can be confirmed, then the potential exists to engineer single cells of whole sheets of functional epithelium for therapeutic purposes.

Tissue engineered scaffolds for rhinologic application

Despite the relative successes in the use of biomaterials in ORL, there remain significant challenges where the desired parameters cannot be met using current materials. Other areas of the field can be said to be still in their infancy. For example, considering ossicular prostheses, whilst biocompatibility parameters are being established, little attention has been addressed to optimizing the acoustic properties of the material used. Significant improvements in implant performance are associated with a corresponding development in materials, but for each new application of a biomaterial, testing to ensure safety and efficacy must be done.

The most difficult and costly development for a new medical implant involves materials that have no history as biomaterials, because of the costly and lengthy process of the preclinical process required for clinical trial accreditation by the American Food and Drug Association and equivalent organizations elsewhere. The development of new implants for use in rhinology requires pro-active engagement by clinicians with bioengineers to highlight potential clinical applications. Areas of ORL which have been problematic for biomaterials include repair of stenotic trachea, replacement of the larynx, total tympanic membrane replacement, accelerated mucosal healing after sinus surgery and reducing fibrous tissue formation after middle ear surgery. Biodegradable implants that promote tissue regeneration would obviate the concern about long-term implant failure due to mechanical mismatch at the implant-tissue interface and show great promise for some of the above applications. For permanent implants, seeding with cells, such as those described above, is increasingly recognized as essential if excessive scarring and stenosis are not to occur.

THE FUTURE

With all the potential that exists in employing stem cells in tissue engineering and regenerative medicine, there still is much to do if the promise of stem cells is to be realized. In spite of all the progress that has been made in understanding the biology of stem cells, there is so much that still needs to be done if we are to be able to not only control, but optimize the differentiation and growth of stem cells. This includes achieving a better understanding of the signals that trigger differentiation, including chemical stimuli, matrix/substrate associated cues, supplying adequate oxygenation and vascularity, and the role of physical factors.

Beyond the science, as one translates stem cell biology into clinical application there are other issues and challenges. First, since there may be a need for a relatively large number of cells,

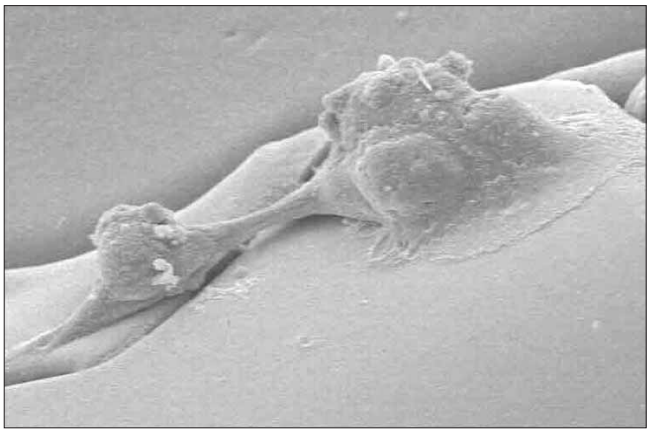

Figure 1. SEM micrograph of a chondrocyte, cultured from nasal cartilage, on a scaffold showing rounded morphology and undergoing mitosis after 24 hours. Reproduced by permission from A. Vats.

how can stem cells be optimally expanded? What types of innovative bioreactor technology will be needed [56]? What kind of process quality control and release criteria (easy for mass produced products, but problematic for individualized treatments typical of first generation RegenMed) will be required if one is to obtain approval from the appropriate regulatory bodies? Secondly, how best to deliver stem cells, to target these cells for therapeutic purposes? For some cases it may be that pure cell implantation will be the best strategy? Even in this case there may be a question as to whether one implants stem cells or differentiated cells?

In many cases, the optimal approach will be to incorporate the cells into a scaffold (see Figure 1), such as that used for the successful tracheal graft [53]. Such a scaffold could be synthetic material, e.g. a polymer, or it could be a natural biological matrix. Just as it is now recognized that cells in a three-dimensional architecture behave differently from cells in monolayer culture, one should expect similar differences for stem cells. As part of this, there are a variety of questions still to be answered. Examples include if a synthetic material, should it be biodegradable? If so, over what time period should it be designed to degrade? For applications not requiring the persistence of high biomechanical strength, the answer will be yes. Should the material incorporate cytokines or growth factors, for example to stimulate angiogenesis or direct *in situ* differentiation of stem cells? The answer to these questions very much depends on the application; however, here again these issues are relatively unexplored. Finally, if off-the-shelf availability is desired for a particular clinical application, then allogeneic or xenogenic cells will need to be employed. This raises the issue of the immunogenicity of the cells for those contexts not involving an immunologically-priveleged site. Everything that we now know suggests that a differentiated cell, one derived from a stem cell, will exhibit the normal immunogenic characteristics of that particular type of differentiated cell. If immune acceptance is an issue, there will then need to be a strategy for overcoming this. Finding the most effective ways of utilizing stem cells, from adult, fetal, and embryonic sources, and triggering their differentiation in a controlled manner will provide cell banks for the *in vitro* growth of tissue and for cell replacement therapy. Developing these concepts from bench to bedside will be crucial in meeting healthcare needs in the coming century.

REFERENCES

1. Vacanti JP, Langer R. Tissue engineering: the design and fabrication of living replacement devices for surgical reconstruction and transplantation. Lancet. 1999; 354, (Suppl 1): 32.
2. Griffiths LG, Naughton G. Tissue engineering--current challenges and expanding opportunities. Science. 2002; 295: 1009.
3. Regenerative Medicine Glossary, Regenerative Medicine. Vol 4, No 4s, S1-S88, DOI 10.2217/rme.09.s1 2009. http://www.futuremedicine.com/toc/rme/4/4s
4. Sanders GT. Amputation Prosthetics, F.A. Davis Company, Philadelphia. 1986.
5. Ratner BD. Applications of Materials in Medicine and Dentistry. Ch. 7 in Ratner BD, Hoffman AS, Schoen FJ, Lemmons JE. (eds), Biomaterials Science, Academic Press, San Diego, 283-388. 1996.
6. Chapekar MS. Tissue Engineering: Challenges and opportunities, J Biomed Mater Res. 2000; 53: 617-620.
7. Spector M. Biomaterial failure. Orthop Clin North Am. 1992; 23: 211-217.
8. Anderson JM. Inflammation and the foreign body response. Prob Gen Surg. 1994; 11: 147.
9. Hutmacher DW. Scaffolds in tissue engineering bone and cartilage. Biomaterials. 2000; 21: 2529-2543.
10. Hench LL. Biomaterials: a forecast for the future. Biomaterials. 1998; 19: 1419-1423.
11. Stock UA, Vacanti JP. Tissue engineering: current state and prospects. Ann Rev Med. 2001; 52: 443–451
12. Asahara T, Murohara T, Sullivan A, et al. Isolation of putative progenitor endothelial cells for angiogenesis. Science. 1997; 275: 964-967.
13. Hirschi KK, Ingram DA, Yoder MC. Assessing identity, phenotype, and fate of endothelial progenitor cells. Arter Throm Vasc Biol. 2008; 28: 1584-1595.
14. Caplan AI. Why are MSCs therapeutic? New data: new insight. J Pathol. 2009; 217: 318-324.
15. Ramasamy R, Tong CK, Seow HF, Vidyadaran S, Dazzi F. The immunosuppressive effects of human bone marrow-derived mesenchymal stem cells target T cell proliferation but not its effector function. Cell Immunol. 2008; 251: 131-136.
16. Newman RE, Yoo D, LeRoux MA, Danilkovitch-Miagkova A. Treatment of inflammatory diseases with mesenchymal stem cells. Inflamm Allergy Drug Targets. 2009; 8: 110-123.
17. Cananzi M, Atala A, De Coppi P. Stem cells derived from amniotic fluid: new potentials in regenerative medicine. Reprod Biomed Online. 2009; 18, Suppl 1: 17-27.
18. Zhang Y, McNeill E, Tian H, et al. Urine derived cells are a potential source for urological tissue reconstruction. J Urol. 2008; 180: 2226-2233.
19. Thomson JA, Itskovitz-Eldor J, Shapiro SS, et al. Embryonic Stem Cell Lines Derived from Human Blastocysts. Science. 1998; 282: 1145-1147.
20. Pickering S, Minger SL, Patel M, et al. Generation of a human embryonic stem cell line encoding the cystic fibrosis mutation deltaF508, using preimplantation genetic diagnosis. Reprod Biomed Online. 2005; 10: 390-397.
21. Takahashi K, Tanabe K, Ohnuki M, et al. Induction of pluripotent stem cells from adult human fibroblasts by defined factors. Cell. 2007; 131: 861-872.
22. Huangfu D, Osafune K, Maehr R, et al. Induction of pluripotent stem cells from primary human fibroblasts with only Oct4 and Sox2. Nat Biotechnol. 2008; 26: 1269-1275.
23. Woltjen K, Michael IP, Mohseni P, et al. PiggyBac transposition reprograms fibroblasts to induced pluripotent stem cells. Nature. 2009; 458: 766-770.
24. Yu J, Hu K, Smuga-Otto K, et al. Human Induced Pluripotent Stem Cells Free of Vector and Transgene Sequences. Science. 2009; 324: 797-801.
25. Aasen T, Raya A, Barrero MJ, et al. Efficient and rapid generation of induced pluripotent stem cells from human keratinocytes. Nat Biotechnol. 2008; 26: 1276-1284.
26. Loh Y, Agarwal S, Park IH, et al. Generation of induced pluripotent stem cells from human blood. Blood. 2009; 113: 5476-5479.
27. Chambers S, Fasano CA, Papapetrou EP, Tomishima M, Sadelain M, Studer L. Highly efficient neural conversion of human ES and iPS cells by dual inhibition of SMAD signaling. Nat Biotechnol. 2009; 27: 275-280.
28. Choi K, Yu J, Smuga-Otto K, et al. Hematopoietic and Endothelial Differentiation of Human Induced Pluripotent Stem Cells. Stem Cells. 2009; 27: 559-567.

29. Taura D, Sone M, Homma K, et al. Induction and isolation of vascular cells from human induced pluripotent stem cells--brief report. Arterioscler Thromb Vasc Biol. 2009; 29: 1100-1103.
30. Taura D, Noguchi M, Sone M, et al. Adipogenic differentiation of human induced pluripotent stem cells: comparison with that of human embryonic stem cells. FEBS Lett. 2009; 583: 1029-1033.
31. Zhang D, Jang W, Liu M, et al. Highly efficient differentiation of human ES cells and iPS cell into mature pancreatic insulin-producing cells. Cell Res 2009; 19: 429-438.
32. Vats A, Bielby RC, Tolley NS, Nerem R, Polak JM. Stem cells. Lancet. 2005; 366(9485): 592-602.
33. Au E, Roskams AJ. Olfactory ensheathing cells of the lamina propria *in vivo* and *in vitro*. Glia. 2003; 41: 224-236.
34. DeLucia TA, Conners JJ, Brown TJ, Cronin CM, Khan T, Jones KJ. Use of a cell line to investigate olfactory ensheathing cell-enhanced axonal regeneration. Anat Rec. 2003; 271B: 61-70.
35. Li Y, Decherchi P, Raisman G. Transplantation of olfactory ensheathing cells into spinal cord lesions restores breathing and climbing. J Neurosci. 2003; 23: 727-731.
36. Grande DA, Mason J, Light E, Dines D. Stem cells as platforms for delivery of genes to enhance cartilage repair. J Bone Joint Surg Am. 2003; 85-A Suppl 2: 111-116.
37. Peng H, Wright V, Usas A, et al. Synergistic enhancement of bone formation and healing by stem cell-expressed VEGF and bone morphogenetic protein-4. J Clin Invest. 2002; 110: 751-759.
38. Jang W, Youngentob SL, Schwob JE. Globose basal cells are required for reconstitution of olfactory epithelium after methyl bromide lesion. J Comp Neurol. 2003; 460: 123-140.
39. Murray RC, Navi D, Fesenko J, Lander AD, Calof AL. Widespread defects in the primary olfactory pathway caused by loss of Mash1 function. J Neurosci. 2003; 23: 1769-1780.
40. Wu HH, Ivkovic S, Murray RC, et al. Autoregulation of neurogenesis by GDF11. Neuron. 2003; 37: 197-207.
41. Calof AL, Bonnin, A, Crocker C, et al. Progenitor cells of the olfactory receptor neuron lineage. Microsc Res Tech. 2002; 58: 176-188.
42. Lipson AC, Widenfalk J, Lindqvist E, Ebendal T, Olson L. Neurotrophic properties of olfactory ensheathing glia. Exp Neurol.2003; 180: 167-171.
43. Doetsch F, Caille I, Lim DA, Garcia-Verdugo JM, Alvarez-Buylla A. Subventricular zone astrocytes are neural stem cells in the adult mammalian brain. Cell. 1999; 97: 703-716.
44. Lois C, Alvarez-Buylla A. Long-distance neuronal migration in the adult mammalian brain. Science. 1994; 264: 1145-1148.
45. Lois C, Garcia-Verdugo JM, Alvarez-Buylla A. Chain migration of neuronal precursors. Science. 1996; 271: 978-981.
46. Tallheden T, Dennis JE, Lennon DP, Sjogren-Jansson E, Caplan AI, Lindahl A. Phenotypic plasticity of human articular chondrocytes. J Bone Joint Surg Am. 2003; 85-A Suppl 2: 93-100.
47. Lindahl A, Brittberg M, Peterson L. Cartilage repair with chondrocytes: clinical and cellular aspects. Novartis Found Symp. 2003; 249: 175-189, 234-241.
48. Peterson L, Minas T, Brittberg M, Lindahl A. Treatment of osteochondritis dissecans of the knee with autologous chondrocyte transplantation: results at two to ten years. J Bone Joint Surg Am. 2003; 85-A Suppl 2: 17-24.
49. Kuriwaka M, Ochi M, Uchio Y, et al. Optimum combination of monolayer and three-dimensional cultures for cartilage-like tissue engineering. Tissue Eng. 2003; 9: 41-49.
50. Ochi M, Uchio Y, Kawasaki K, Wakitani S, Iwasa J. Transplantation of cartilage-like tissue made by tissue engineering in the treatment of cartilage defects of the knee. J Bone Joint Surg Br. 2002; 84: 571-578.
51. Lavezzi A, Mantovani M, della Berta LG, Matturri L. Cell kinetics of human nasal septal chondrocytes *in vitro*: importance for cartilage grafting in otolaryngology. J Otolaryngol. 2002; 31: 366-370.
52. Brittberg M, Peterson L, Sjogren-Jansson E, Tallheden T, Lindahl A. Articular cartilage engineering with autologous chondrocyte transplantation. A review of recent developments. J Bone Joint Surg Am. 2003; 85-A Suppl 3: 109-115.
53. Macchiarini P, Jungebluth P, Go T, et al. Clinical transplantation of a tissue-engineered airway. Lancet. 2008; 372(9655): 2023-2030.
54. Vats A, Bielby R, Tolley NS, et al. Chondrogenic differentiation of Human Embryonic Stem Cells: The effect of the Micro-Environment. Tissue Eng. 2006; 12: 1687-1697.
55. Snyder JC, Teisanu RM, Stripp BR. Endogenous lung stem cells and contribution to disease. J Pathol. 2009; 217: 254-264.
56. Asnaghi A, Macchiarini P, Mantero S. Tissue engineering toward organ replacement: a promising approach in airway transplant. Int J Artif Organs. 2009; 32: 763-768.

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