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Stem Cells in Plastic Surgery: A Review of Current Clinical and Translational Applications

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Background Stem cells are a unique cell population characterized by self-renewal and cellular differentiation capabilities. These characteristics, among other traits, make them an attractive option for regenerative treatments of tissues defects and for aesthetic procedures in plastic surgery. As research regarding the isolation, culture and behavior of stem cells has progressed, stem cells, particularly adult stem cells, have shown promising results in both translational and clinical applications.

Methods The purpose of this review is to evaluate the applications of stem cells in the plastic surgery literature, with particular focus on the advances and limitations of current stem cell therapies. Different key areas amenable to stem cell therapy are addressed in the literature review; these include regeneration of soft tissue, bone, cartilage, and peripheral nerves, as well as wound healing and skin aging.

Results The reviewed studies demonstrate promising results, with favorable outcomes and minimal complications in the cited cases. In particular, adipose tissue derived stem cell (ADSC) transplants appear to provide effective treatment options for bony and soft tissue defects, and non-healing wounds. ADSCs have also been shown to be useful in aesthetic surgery.

Conclusions Further studies involving both the basic and clinical science aspects of stem cell therapies are warranted. In particular, the mechanism of action of stem cells, their interactions with the surrounding microenvironment and their long-term fate require further elucidation. Larger randomized trials are also necessary to demonstrate the continued safety of transplanted stem cells as well as the efficacy of cellular therapies in comparison to the current standards of care.

Keywords Stem cells / Mesenchymal stem cells / Clinical trial

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INTRODUCTION

Stem cells are a unique population of undifferentiated biological cells that have the capacity to self-renew and differentiate into different cell types. They play a central role in the field of regenerative medicine, aimed at the repair and replacement of diseased cells, tissues and organs through the transplantation of healthy cells and tissues; in particular, stem cells [1]. Plastic surgery shares several of the same principles with regenerative medicine, historically functioning on a more macroscopic level by using a patient's own tissue to restore and enhance the body. As our understanding of cellular regenerative therapies progresses, plastic surgeons may soon have the option of utilizing a single autologous cellular source for the regeneration of differ-

ent tissue types.

There are several different types of stem cells that have been considered for clinical applications. Embryonic stem cells (ESCs) have the greatest regenerative "potential" being that they are naturally pluripotent and can differentiate into all adult cellular types. The successful isolation and culture of human ESCs has allowed investigators to gain a much better understanding of the capabilities of these cells to regenerate different tissue types [2]. ESC research, however, has been restricted by controversy surrounding the origin and isolation of these cells. Additional obstacles include safety concerns over potential tumorigenicity [3] and immunocompatibilty [4]. These issues, as well as the ethical barriers have significantly limited the clinical applicability of ESCs at this time.

Adult stem cells such as mesenchymal stem cells (MSCs) circumvent many of the ethical and technical issues associated with ESCs as they can be isolated from developed tissues including bone marrow, fat, and skin (bone marrow stromal cells [BMSCs], adipose tissue-derived stem cells [ADSCs], and adult skin stromal cells [ASSCs], respectively) [5]. However, these cells are multipotent, and are therefore restricted to the cell lineage in which they reside. Regardless, adult stem cells are a highly useful cell population in regenerative medicine as their ease of isolation, multilineage differentiation, and potential for autologous transplantation makes them a favorable candidate for clinical translation.

The creation of induced pluripotent stem cell (iPSC) lines, or adult somatic cells reprogrammed into pluripotent cells, has allowed researchers to utilize the differentiation capabilities of ESCs, while avoiding the ethical issues associated with ESC isolation. iPSCs share many similar properties with ESCs including expression of certain stem cells genes and proteins, chromatin methylation patterns, potency and differentiability [6]. Importantly, iPSCs can be created from several different, easily accessible cell types [7-10]. However, clinical translations of iPSC therapies still have noteworthy challenges. Generation of iPSCs has a low reprogramming efficiency [11] and requires the introduction of exogenous transcription factors with viral vectors [6] or through other significant *ex vivo* manipulations of cells [12,13]. This process has led to concerns over the stability of these cell lines [14] and the possibility of chromosomal aberrations [15], preventing safe use in human trials currently.

ADSCs have recently been investigated as a source of multilineage precursor cells [16], and are particularly promising for regenerative therapies as they can be easily harvested with minimal donor site morbidity [17]. In addition, ADSCs have a differentiation potential similar to other MSCs as well as a higher yield upon isolation and a greater proliferative rate in culture when compared to BMSCs [18-20]. The discovery that ADSCs are not only precursor to adipocytes, but are multipotent progenitors to a variety of cells [21] was a milestone that has allowed scientists to utilize the true potential of ADSCs to derive several additional cell types including osteoblasts, chondrocytes, myocytes, epithelial cells and neuronal cells [22]. For the plastic surgeon, they are an abundant source of multipotent stem cells that can be easily accessed during many routine procedures.

Stem cells are a promising therapeutic modality for the treatment of tissue defects, malformations and disease, and an attractive tool for the enhancement of aesthetic medicine. However, scientific evidence on clinical applications is still limited and much is unknown about the safety and efficacy of stem cell therapies [23]. Several key issues must be considered including the 1) source of stem cells, 2) efficiency of transplantation, 3) engraftment in host tissue, 4) interaction with the surrounding microenvironment, and 5) long term fate of transplanted cells. By further elucidating the current strategies for stem cell utilization, this review aims to provide a better understanding of the current state of cellular regenerative techniques in plastic surgery, the progress that remains to be made, and the appropriate direction for future research.

SOFT TISSUE AUGMENTATION AND REGENERATION

The regeneration and augmentation of soft tissue requires the restoration and enhancement of form as well as the continued long-term maintenance of aesthetic results. Current therapies are limited, and include biomaterials, which can be complicated by infection, surrounding fibrosis, and contracture and are also associated with high cost. Other viable options include composite tissue flaps as well as transplantation of autologous fat to fill defects, or fat grafting [24]. Fat grafting is a commonly performed procedure for soft tissue filling that can be used for several indications including facial lipodystrohpy, lower limb atrophy, and breast augmentation and reconstruction [25]. Autologous fat utilized in fat grafting contains a variety of cells, including ADSCs [26], which are well suited to support regeneration of tissue as their ability to secrete angiogenic growth factors such as vascular endothelial growth factor (VEGF) [27] promotes neo-vascularization of new tissues [28]. Fat is usually harvested and finely divided simultaneously, as in suction harvesting, or sequentially harvested and subsequently separated by mechanical means and/or enzymatic digestion to then be reintroduced by injection. Though this procedure is widely used among plastic surgeons, there remains a lack of standardization for harvesting, processing and reinjection protocols, and

the universal principles that underlie successful application of lipoinjection have yet to be determined.

Fat grafts, however, are restricted by varying rates of resorption [29] and complications of partial necrosis, resulting in unreliable long-term outcomes after transplantation [30]. Cell-assisted lipotransfer (CAL) is a technique that combines aspirated fat with concentrated ADSCs in the stromal vascular fraction (SVF) of lipoaspirate to create ADSC-rich fat grafts. This approach allows for marked improvements in the survival rate of transplanted fat as well as a decrease in adverse effects of lipoinjection such as fibrosis and cyst formation [31]. In 2008, Yoshimura et al. [32] used CAL for cosmetic breast augmentation in forty patients with reported increases in breast circumference in all patients at six months and no major complications. Other studies utilizing CAL for cosmetic breast augmentation have also reported increased breast volumes with improved contour and minimal complications [33-35]. CAL has also been used for facial lipoatrophy [36,37], as well as for facial augmentation during face-lift and facial contouring surgeries, with similar noted subjective clinical improvements [38]. In addition, a study quantifying fat graft volumes with computed tomography scans showed that fat grafts with concentrated ADSCs underwent less reabsorption than fat grafts alone in ten patients with hemifacial atrophy [39]. These preliminary studies suggest that ADSCs might allow for improvements in the retention and volume-restoring capabilities of transplanted fat. However, a well-controlled clinical trial comparing these two modalities with respect to enhancement and retention of aesthetic results will ultimately be necessary to draw appropriate conclusions.

Alternative ADSC therapies have also been investigated for soft tissue regeneration. Kim et al. [40] use immature adipocytes differentiated from ADSCs *in vitro* for the treatment of depressed scars, with up to 75% recovery in volume of scars at twelve weeks. Other ADSC preparations include stem cell-enriched tissue (SET) injections [41], in which isolated autologous ADSCs are injected into the area of a patient's body that received traditional fat grafts earlier that day [42]. Advantages of this model include reduced time spent in the operating room and therefore decreased procedure cost compared to CAL. Studies comparing the two techniques, however, are not available, and larger, randomized trials with both techniques will be necessary to determine efficacy.

Of note, the Food and Drug Administration (FDA) recently issued a statement declaring that autologous adipose stem cells from SVF are considered to be a "drug" due to the use of collagenase during component separation, and must therefore be completely regulated by the FDA [43,44]. In practical terms, any surgeon that wishes to use SVF must submit an investigational new drug (IND) application and have an approved Institutional Review Board (IRB), a costly and time-consuming process. Further investigation into other methods of separating cells and stroma such as mechanical separation by centrifugation and rapid isolation techniques may be warranted given the implications of these new regulations.

BONY RECONSTRUCTION

Autologous bone grafts have been the gold standard for reconstructing bony defects [45], but donor site morbidity [46] and complications associated with alternatives such as alloplastic implants [47] have led researchers to investigate cell-based therapies. Both BMSCs and ADSCs have proven to be favorable candidates based on their osteogenic capacity in *in vitro* and *in vivo* studies [21,48-50].

Current clinical stem cell therapies for bone regeneration have shown promising results for craniofacial defects [51-55]. Calvarial defects in particular have been a specific area of focus due to the unqiue challenges with repair as the calvarium is unable to ossify on its own in patients over two years old [46], and as the size of these defects is often greater than the amount of autologous bone available in the pediatric population [51]. ADSCs have been combined with milled autologous cancellous bone and fibrin glue to repair a large calvarial defect with resulting new bone formation and near complete ossification of the preoperative defect at three months [51]. However, the utilization of multiple concomitant treatments limits the ability to comprehend the degree of the therapeutic effect of ADSCs in this study. In a more recent study, Thesleff et al. [53] transplant ADSCs seeded in β-tricalcium phosphate (TCP) granules to successfully repair critical size calvarial defects (65–90 mm × 37–75 mm) in four patients without the use of autologous bone grafting. Using CT scans to quantify ossification, the authors demonstrate that the Hounsfield units of ADSC cranioplasties approach those of surrounding intact bone. These results suggest that ADSCs alone are capable of appropriately ossifying defects without the use of exogenous growth factors, and therefore provide a relatively simple method of autologous bony reconstruction with little donor site morbidity.

Stem cell treatments have also been used for repair of defects involving the maxilla and mandible. Certain approaches, with both ADSC [52] and BMSC [54,55] transplants, utilize a multistep procedure in which harvested stem cells are combined with different growth factors (bone morphogenetic protein [BMP]-2 [52] and BMP-7 [54,55]) in a scaffold, and are then re-implanted into the patient's muscle tissue to allow for ectopic bone formation. In a third procedure, occurring seven [54,55] to nine

months [52] after implantation, the titanium-enclosed ectopic bone is transplanted with the surrounding muscle and vascular pedicle as a composite microvascular flap to fill the bony defect. This technique has yielded excellent functional and aesthetic results; however, it is fairly complex and requires multiple different procedures staged over the period of several months. Sandor et al. [56] propose a 1-stage procedure in which harvested ADSCs seeded on a scaffold of β-TCP and BMP-2 are placed in a molded titanium mesh to fill a mandibular defect. This protocol, named *in situ bone formation*, circumvents the need for ectopic bone formation and for a second surgical site, while producing favorable clinical outcomes as well as histologic signs of bone formation and remodeling at ten months after transplant. However, until the mechanisms behind osteogenic transformation can be further elucidated and the degree of ossification better quantified, it will be difficult to compare the efficacy of different cell-based treatments.

CARTILAGE FORMATION

Cartilage defects present a challenging reconstructive problem due to the tissue's limited intrinsic capacity for self-repair. Currently, the only FDA-approved cellular-based therapy for cartilage defects involves autologous chondrocyte implantation (ACI), in which chondrocytes harvested from low-contact areas are expanded in culture and then re-injected into a defect [57]. This technique has shown promising results in early clinical studies [57], but is restricted by limited expansion of chondrocytes *ex vivo*, difficulty maintaining chondrocyte phenotype *in vitro*, and donor site morbidity [58,59]. Alternative cellular therapies have turned to progenitor cell populations such as BMSCs, which have the ability to differentiate into several connective tissue cells types, including cartilage [60]. Clinically, autologous BMSCs have been used to repair articular cartilage defects by surgically transplanting collagen-embedded BMSCs [61-63] and by intra-articular injections of BMSCs [64]. Both techniques have yielded promising results with noted improvements in clinical symptoms such as pain and walking ability.

ADSCs have also been investigated as a less invasive source of chondrocyte progenitors that can be differentiated into chondrocytes *in vitro* [16]. Important considerations in this process include the use of appropriate growth factors, primarily those in the TGF-β superfamily [65], as well culture in a 3-dimensional environment by utilizing cellular scaffolds [66]. These preconditioned ADSCs are then capable of forming cartilage tissue *in vivo* [67]. In addition, uninduced ADSCs transplanted into hyaline cartilage defects in patellofemoral joints [68] and ear auricle defects [69] in animals have completely restored the native cartilage structure and fully repaired the defects at six months and three months, respectively. Limiting the *ex vivo* manipulation of these cells provides a more favorable technique for future clinical applications and demonstrates the intrinsic ability of ADSCs to adapt to their environment *in vivo* without the need for exogenous growth factors and substrates pre-transplantation.

WOUND HEALING

Wound healing is a highly coordinated process involving complex interactions among cells, growth factors and extracellular matrix (ECM) molecules to sequentially achieve hemostasis, cell proliferation, angiogenesis, re-epithelialization and remodeling of tissue. ADSCs have been promoted as favorable candidates for wound therapies as they secrete numerous growth factors and cytokines critical in wound healing [70,71] and also increase macrophage recruitment, enhance granulation tissue, and improve vascularization [72,73]. These reparative capabilities are illustrated in a study by Rigotti et al. [74], which examines the role of ADSCs in treating severe (LENT-SOMA grade 3) and irreversible (LENT-SOMA grade 4) radiation-induced lesions with atrophy, fibrosis, ulceration and retraction. Repeated transplants of purified autologous lipoaspirates into irradiated areas resulted in improvement of ultrastructural tissue characteristics with neovessel formation as well as significant clinical improvements with the majority of patients exhibiting a decrease in LENT-SOMA scores to 0 or 1. Similar results have been reported in animal models of radiation injury with increased vessel density in wounds treated with ADSCs [72,75]. These studies also elucidate possible reparative mechanisms of ADSCs such as the release of keratinocyte growth factor [72] and the differentiation of ADSCs toward endothelial and epithelial phenotypes [72,75]. Akita et al. report a case of an intractable sacro-coccygeal radiation ulcer treated with autologous ADSCs, artificial dermis, and basic fibroblast growth factor that healed uneventfully by 82 days after initial treatment. Similar mechanisms may have be responsible for improved healing in this case though it is difficult to determine due to the administration of multiple treatment modalities.

The angiogenic properties of ADSCs may also be beneficial in other wounds complicated by ischemia, such as in the setting of critical limb ischemia. Lee et al. [76] utilize intramuscular injections of ADSCs to treat patients with thromboangiitis obliterans and diabetic feet with improvement in pain rating scores in the majority of patients as well as improved walking distances measured in a subset of patients. Similar to observations of neovessel formation by Rigotti et al. [74], transplantation of ADSCs into ischemic limbs increased blood flow as seen by new collateral

vessel formation using digital subtraction angiography at six months after transplant. Autologous transplantation of other cell types such as BMSCs have also shown promising results in limb ischemia patients, including evidence of increased collateral vessels, improved ankle-brachial index and transcutaneous oxygen pressure, and improvements in patients' walking distance and resting pain after cell transplantation [77-79]. However, it should be noted that adverse affects such as unfavorable angiogenesis have been noted in patients receiving bone marrow mononuclear cells [80] and that clinical improvements can vary depending on the underlying cause of critical limb ischemia [81]. Though Lee et al. [76] report no complications following their transplantations, utilization of these therapies should proceed with caution as they are still novel and their effects not completely understood.

ADSCs may also be suited for the treatment of pathological wound healing in the context of aberrant scar formation. The extent of scar formation is closely associated with the inflammatory process in wound healing [82], providing a potential therapeutic target for excessive scarring as ADSCs have been shown to have anti-inflammatory and immunosuppressive effects [83,84]. Yun et al. [85] injected ADSCs subcutaneously into scars formed from full thickness skin defects on the backs of pigs . Their results showed that scar surface area was significantly smaller in the experimental group, which also had greater improvements in scar color and pliability. Interestingly, scars injected with ADSCs also had fewer mast cells, possibly decreasing fibroblast proliferation and supporting the concept of inflammatory modulation in controlling scar formation. As scar formation is an essential part of normal wound healing, it will be important for future therapies to regulate the modulation of inflammatory processes to achieve an appropriate balance between necessary and excessive scar formation.

SKIN REJUVENATION

Skin aging involves a number of different degenerative processes, notably a decrease in collagen production by fibroblasts. Several cytokines and growth factors are involved in stimulating fibroblast collagen synthesis for skin rejuvenation, and have also been shown to be part of the secretome of ADSCs [86], suggesting that these cells may be suitable for promoting repair of atrophic and photo-damaged skin. Animal studies have shown that subcutaneous ADSC injections increase dermal thickness and collagen density in aged mice [87], and reduce wrinkles induced by UVB-irradiation [88]. Suggested mechanisms include paracrine activation of dermal fibroblasts and dermal angiogenesis [87,89]. In a clinical pilot study, Park et al. [86] injected autologous lipoaspirate (PLA) cells containing approximately 20% to 30% ADSCs intradermally in the photo-aged aged skin of one patient. They reported an improvement in general skin texture and wrinkles after two months as well as an increase in dermal thickness by ultrasonography. These promising outcomes are similar to the results of translational studies, though further elucidation of the mechanisms behind these effects is necessary prior to further applying these therapies.

PERIPHERAL NERVE REGENERATION

The repair of peripheral nerve injuries (PNIs), particularly those with large defects, is limited by donor site morbidity and suboptimal functional recovery, prompting research for alternative treatments that have included a wide spectrum of regenerative therapies. A majority of experimental stem cell treatments for PNI focus on replacing host support cells, particularly the Schwann-cell population, as these cells are crucial in providing trophic, structural and directional support for regenerating axons. Neural stem cells are a logical choice as natural precursors to Schwann cells (SCs), and improve regeneration in animal models of PNI [90]. However, they are restricted by difficulties with isolation as well as ethical problems. ESCs have likewise been used to promote nerve repair in animals [91], but are currently limited by similar issues.

Adult stem cells such as BMSCs are a useful source of autologous cells that are multipotent, but can be trans-differentiated into SC-like cells [92]. ADSCs also have the capacity to replace host SCs [17] and can promote nerve regeneration when differentiated into neuronal-like lineages as well [93]. In addition, these cells are more easily accessible than BMSCs and are comparable to BMSCs in their capacity to promote peripheral nerve regeneration in animals [94]. The skin serves as another reliably accessible source of stem cells. A population of undifferentiated adult stem cells can be found in the hair follicle bulge, and has been differentiated into several cell types including neuronal-like [95] and SC-like cells [96]. The dermis also contains neural crest precursor cells that have been shown to improve nerve regeneration in the chronically denervated nerve [97].

Alternative approaches to stem cell-mediated peripheral nerve regeneration focus on modulating the nerve injury niche to provide trophic support for host cells. Transplants of undifferentiated ADSCs into peripheral nerve injuries have demonstrated that ADSCs can secrete several neurotrophic factors such as nerve growth factor, glial cell line-derived neurotrophic factor and brain-derived neurotrophic factor *in vivo* [98,99]. In addition, ADSCs express genes that belong to the glial phenotype

and are responsible for neuron metabolism and function [100]. These findings suggest that ADSCs may be particularly suited to create a favorable environment to support regenerating axons. However, the overall mechanism of action behind ADSCs' influence on nerve regeneration is still relatively unknown. Moving forward with translational studies will require a better definition of the role of ADSCs as a paracrine influence that promotes regeneration in the surrounding tissue or as a progenitor cell that replaces host tissues.

CONCLUSIONS

Regenerative medicine has made significant progress over the last several years with regards to further understanding stem cell biology and the different applications of stem cells for the treatment of clinical problems. The field of plastic surgery is no exception, and stem cells have been reported to be effective in treating a variety of defects including bony and soft tissue defects, as well as non-healing wounds complicated by radiation and ischemia. Aesthetic procedures such as breast augmentation and skin rejuvenation have also shown positive outcomes with stem cell treatments. Importantly, these studies have noted minimal complications from these cell-based therapies. ADSCs have proven to be particularly useful as their ease of isolation and efficient *ex vivo* culture makes them favorable candidates for clinical applications. However, much remains unknown about the mechanisms of action behind the therapeutic effects of these cells. In this regard, it may be beneficial for future efforts to focus on further investigating the survival of transplanted cells in the injury niche, the controlled proliferation of stem cells after transplantation, and the appropriate integration of the transplanted cells into their surrounding environment.

In addition, the majority of the clinical literature is comprised of case reports and small case series. These cases are valuable studies for creating a foundation to direct future experiments; however, large scale, randomized trials will eventually be necessary to determine the true safety and efficacy of these novel treatments. Overall, the recent clinical advances in stem cell therapies suggest a promising future for regenerative medical therapies in plastic surgery. However, as the basic science of stem cell behavior continues to be revealed, cautious and controlled implementation of cell-based therapies will be crucial for the appropriate translation of this new technology to the clinical setting.

REFERENCES

1. Walia B, Satija N, Tripathi RP, et al. Induced pluripotent stem cells: fundamentals and applications of the reprogramming process and its ramifications on regenerative medicine. Stem Cell Rev 2012;8:100-15.
2. Thomson JA, Itskovitz-Eldor J, Shapiro SS, et al. Embryonic stem cell lines derived from human blastocysts. Science 1998;282:1145-7.
3. Ben-David U, Benvenisty N. The tumorigenicity of human embryonic and induced pluripotent stem cells. Nat Rev Cancer 2011;11:268-77.
4. Swijnenburg RJ, Schrepfer S, Govaert JA, et al. Immunosuppressive therapy mitigates immunological rejection of human embryonic stem cell xenografts. Proc Natl Acad Sci U S A 2008;105:12991-6.
5. Al-Nbaheen M, Vishnubalaji R, Ali D, et al. Human stromal (mesenchymal) stem cells from bone marrow, adipose tissue and skin exhibit differences in molecular phenotype and differentiation potential. Stem Cell Rev 2013;9:32-43.
6. Takahashi K, Yamanaka S. Induction of pluripotent stem cells from mouse embryonic and adult fibroblast cultures by defined factors. Cell 2006;126:663-76.
7. Lowry WE, Richter L, Yachechko R, et al. Generation of human induced pluripotent stem cells from dermal fibroblasts. Proc Natl Acad Sci U S A 2008;105:2883-8.
8. Hanna J, Markoulaki S, Schorderet P, et al. Direct reprogramming of terminally differentiated mature B lymphocytes to pluripotency. Cell 2008;133:250-64.
9. Aasen T, Raya A, Barrero MJ, et al. Efficient and rapid generation of induced pluripotent stem cells from human keratinocytes. Nat Biotechnol 2008;26:1276-84.
10. Takenaka C, Nishishita N, Takada N, et al. Effective generation of iPS cells from CD34+ cord blood cells by inhibition of p53. Exp Hematol 2010;38:154-62.
11. Phanthong P, Raveh-Amit H, Li T, et al. Is aging a barrier to reprogramming? Lessons from induced pluripotent stem cells. Biogerontology 2013.
12. Gonzalez F, Barragan Monasterio M, Tiscornia G, et al. Generation of mouse-induced pluripotent stem cells by transient expression of a single nonviral polycistronic vector. Proc Natl Acad Sci U S A 2009;106:8918-22.
13. Okita K, Nakagawa M, Hyenjong H, et al. Generation of mouse induced pluripotent stem cells without viral vectors. Science 2008;322:949-53.
14. Steinemann D, Gohring G, Schlegelberger B. Genetic instability of modified stem cells-a first step towards malignant transformation? Am J Stem Cells 2013;2:39-51.
15. Mayshar Y, Ben-David U, Lavon N, et al. Identification and classification of chromosomal aberrations in human induced pluripotent stem cells. Cell Stem Cell 2010;7:521-31.

16. Zuk PA, Zhu M, Ashjian P, et al. Human adipose tissue is a source of multipotent stem cells. Mol Biol Cell 2002; 13:4279-95.
17. di Summa PG, Kalbermatten DF, Pralong E, et al. Long-term *in vivo* regeneration of peripheral nerves through bioengineered nerve grafts. Neuroscience 2011;181:278-91.
18. Higuchi A, Chuang C-W, Ling Q-D, et al. Differentiation ability of adipose-derived stem cells separated from adipose tissue by a membrane filtration method. J Memb Sci 2011; 366:286-94.
19. Gimble JM, Guilak F, Bunnell BA. Clinical and preclinical translation of cell-based therapies using adipose tissue-derived cells. Stem Cell Res Ther 2010;1:19.
20. Strem BM, Hicok KC, Zhu M, et al. Multipotential differentiation of adipose tissue-derived stem cells. Keio J Med 2005; 54:132-41.
21. Zuk PA, Zhu M, Mizuno H, et al. Multilineage cells from human adipose tissue: implications for cell-based therapies. Tissue Eng 2001;7:211-28.
22. Brayfield C, Marra K, Rubin JP. Adipose stem cells for soft tissue regeneration. Handchir Mikrochir Plast Chir 2010; 42:124-8.
23. Gir P, Oni G, Brown SA, et al. Human adipose stem cells: current clinical applications. Plast Reconstr Surg 2012; 129:1277-90.
24. Eremia S, Newman N. Long-term follow-up after autologous fat grafting: analysis of results from 116 patients followed at least 12 months after receiving the last of a minimum of two treatments. Dermatol Surg 2000;26:1150-8.
25. Gir P, Brown SA, Oni G, et al. Fat grafting: evidence-based review on autologous fat harvesting, processing, reinjection, and storage. Plast Reconstr Surg 2012;130:249-58.
26. Brown SA, Levi B, Lequeux C, et al. Basic science review on adipose tissue for clinicians. Plast Reconstr Surg 2010; 126:1936-46.
27. Salgado AJ, Reis RL, Sousa NJ, et al. Adipose tissue derived stem cells secretome: soluble factors and their roles in regenerative medicine. Curr Stem Cell Res Ther 2010;5:103-10.
28. Sheng L, Yang M, Li H, et al. Transplantation of adipose stromal cells promotes neovascularization of random skin flaps. Tohoku J Exp Med 2011;224:229-34.
29. Cherubino M, Marra KG. Adipose-derived stem cells for soft tissue reconstruction. Regen Med 2009;4:109-17.
30. Sommer B, Sattler G. Current concepts of fat graft survival: histology of aspirated adipose tissue and review of the literature. Dermatol Surg 2000;26:1159-66.
31. Matsumoto D, Sato K, Gonda K, et al. Cell-assisted lipotransfer: supportive use of human adipose-derived cells for soft tissue augmentation with lipoinjection. Tissue Eng 2006;12:3375-82.
32. Yoshimura K, Sato K, Aoi N, et al. Cell-assisted lipotransfer for cosmetic breast augmentation: supportive use of adipose-derived stem/stromal cells. Aesthetic Plast Surg 2008; 32:48-55.
33. Kamakura T, Ito K. Autologous cell-enriched fat grafting for breast augmentation. Aesthetic Plast Surg 2011;35:1022-30.
34. Wang L, Lu Y, Luo X, et al. Cell-assisted lipotransfer for breast augmentation: a report of 18 patients. Zhonghua Zheng Xing Wai Ke Za Zhi 2012;28:1-6.
35. Yoshimura K, Asano Y, Aoi N, et al. Progenitor-enriched adipose tissue transplantation as rescue for breast implant complications. Breast J 2010;16:169-75.
36. Yoshimura K, Sato K, Aoi N, et al. Cell-assisted lipotransfer for facial lipoatrophy: efficacy of clinical use of adipose-derived stem cells. Dermatol Surg 2008;34:1178-85.
37. Castro-Govea Y, De La Garza-Pineda O, Lara-Arias J, et al. Cell-assisted lipotransfer for the treatment of parry-romberg syndrome. Arch Plast Surg 2012;39:659-62.
38. Lee SK, Kim DW, Dhong ES, et al. Facial soft tissue augmentation using autologous fat mixed with stromal vascular fraction. Arch Plast Surg 2012;39:534-9.
39. Koh KS, Oh TS, Kim H, et al. Clinical application of human adipose tissue-derived mesenchymal stem cells in progressive hemifacial atrophy (Parry-Romberg disease) with microfat grafting techniques using 3-dimensional computed tomography and 3-dimensional camera. Ann Plast Surg 2012; 69:331-7.
40. Kim M, Kim I, Lee SK, et al. Clinical trial of autologous differentiated adipocytes from stem cells derived from human adipose tissue. Dermatol Surg 2011;37:750-9.
41. Zhu M, Zhou Z, Chen Y, et al. Supplementation of fat grafts with adipose-derived regenerative cells improves long-term graft retention. Ann Plast Surg 2010;64:222-8.
42. Tiryaki T, Findikli N, Tiryaki D. Staged stem cell-enriched tissue (SET) injections for soft tissue augmentation in hostile recipient areas: a preliminary report. Aesthetic Plast Surg 2011;35:965-71.
43. US Food and Drug Administration (FDA). CFR-code of federal regulations title 21. Part 860: medical device classification procedures [Internet]. Silver Spring, MD: FDA; 2013 [2013 Aug 29]. Available from: http://www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfcfr/cfrsearch.cfm?cfrpart=25&showfr=1.
44. Rodriguez RL. FDA: stem cells from your own fat are a drug [Internet]. Lutherville-Timonium, MD: Cosmeticsurg.net; 2013 [2013 Aug 28]. Available from: http://www.cosmetic-

surg.net/blog/2012/01/11/fda-stem-cells-from-your-own-fat-are-a-drug/.
45. Pogrel MA, Podlesh S, Anthony JP, et al. A comparison of vascularized and nonvascularized bone grafts for reconstruction of mandibular continuity defects. J Oral Maxillofac Surg 1997;55:1200-6.
46. Sandor GK, Nish IA, Carmichael RP. Comparison of conventional surgery with motorized trephine in bone harvest from the anterior iliac crest. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 2003;95:150-5.
47. Levi B, Glotzbach JP, Wong VW, et al. Stem cells: update and impact on craniofacial surgery. J Craniofac Surg 2012; 23:319-22.
48. Wadagaki R, Mizuno D, Yamawaki-Ogata A, et al. Osteogenic induction of bone marrow-derived stromal cells on simvastatin-releasing, biodegradable, nano- to microscale fiber scaffolds. Ann Biomed Eng 2011;39:1872-81.
49. Cowan CM, Shi YY, Aalami OO, et al. Adipose-derived adult stromal cells heal critical-size mouse calvarial defects. Nat Biotechnol 2004;22:560-7.
50. Haynesworth SE, Goshima J, Goldberg VM, et al. Characterization of cells with osteogenic potential from human marrow. Bone 1992;13:81-8.
51. Lendeckel S, Jodicke A, Christophis P, et al. Autologous stem cells (adipose) and fibrin glue used to treat widespread traumatic calvarial defects: case report. J Craniomaxillofac Surg 2004;32:370-3.
52. Mesimaki K, Lindroos B, Tornwall J, et al. Novel maxillary reconstruction with ectopic bone formation by GMP adipose stem cells. Int J Oral Maxillofac Surg 2009;38:201-9.
53. Thesleff T, Lehtimaki K, Niskakangas T, et al. Cranioplasty with adipose-derived stem cells and biomaterial: a novel method for cranial reconstruction. Neurosurgery 2011; 68:1535-40.
54. Warnke PH, Springer IN, Wiltfang J, et al. Growth and transplantation of a custom vascularised bone graft in a man. Lancet 2004;364:766-70.
55. Warnke PH, Wiltfang J, Springer I, et al. Man as living bioreactor: fate of an exogenously prepared customized tissue-engineered mandible. Biomaterials 2006;27:3163-7.
56. Sandor GK, Tuovinen VJ, Wolff J, et al. Adipose stem cell tissue-engineered construct used to treat large anterior mandibular defect: a case report and review of the clinical application of good manufacturing practice-level adipose stem cells for bone regeneration. J Oral Maxillofac Surg 2013; 71:938-50.
57. Brittberg M, Lindahl A, Nilsson A, et al. Treatment of deep cartilage defects in the knee with autologous chondrocyte transplantation. N Engl J Med 1994;331:889-95.
58. Huselstein C, Li Y, He X. Mesenchymal stem cells for cartilage engineering. Biomed Mater Eng 2012;22:69-80.
59. Diaz-Romero J, Gaillard JP, Grogan SP, et al. Immunophenotypic analysis of human articular chondrocytes: changes in surface markers associated with cell expansion in monolayer culture. J Cell Physiol 2005;202:731-42.
60. Pittenger MF, Mackay AM, Beck SC, et al. Multilineage potential of adult human mesenchymal stem cells. Science 1999;284:143-7.
61. Nejadnik H, Hui JH, Feng Choong EP, et al. Autologous bone marrow-derived mesenchymal stem cells versus autologous chondrocyte implantation: an observational cohort study. Am J Sports Med 2010;38:1110-6.
62. Wakitani S, Mitsuoka T, Nakamura N, et al. Autologous bone marrow stromal cell transplantation for repair of full-thickness articular cartilage defects in human patellae: two case reports. Cell Transplant 2004;13:595-600.
63. Wakitani S, Nawata M, Tensho K, et al. Repair of articular cartilage defects in the patello-femoral joint with autologous bone marrow mesenchymal cell transplantation: three case reports involving nine defects in five knees. J Tissue Eng Regen Med 2007;1:74-9.
64. Orozco L, Munar A, Soler R, et al. Treatment of knee osteoarthritis with autologous mesenchymal stem cells: a pilot study. Transplantation 2013;95:1535-41.
65. Hennig T, Lorenz H, Thiel A, et al. Reduced chondrogenic potential of adipose tissue derived stromal cells correlates with an altered TGFbeta receptor and BMP profile and is overcome by BMP-6. J Cell Physiol 2007;211:682-91.
66. Estes BT, Diekman BO, Gimble JM, et al. Isolation of adipose-derived stem cells and their induction to a chondrogenic phenotype. Nat Protoc 2010;5:1294-311.
67. Lin Y, Luo E, Chen X, et al. Molecular and cellular characterization during chondrogenic differentiation of adipose tissue-derived stromal cells in vitro and cartilage formation *in vivo*. J Cell Mol Med 2005;9:929-39.
68. Zhang HN, Li L, Leng P, et al. Uninduced adipose-derived stem cells repair the defect of full-thickness hyaline cartilage. Chin J Traumatol 2009;12:92-7.
69. Bahrani H, Razmkhah M, Ashraf MJ, et al. Differentiation of adipose-derived stem cells into ear auricle cartilage in rabbits. J Laryngol Otol 2012;126:770-4.
70. Rehman J, Traktuev D, Li J, et al. Secretion of angiogenic and antiapoptotic factors by human adipose stromal cells. Circulation 2004;109:1292-8.
71. Wang M, Crisostomo PR, Herring C, et al. Human progenitor cells from bone marrow or adipose tissue produce

VEGF, HGF, and IGF-I in response to TNF by a p38 MAPK-dependent mechanism. Am J Physiol Regul Integr Comp Physiol 2006;291:R880-4.
72. Ebrahimian TG, Pouzoulet F, Squiban C, et al. Cell therapy based on adipose tissue-derived stromal cells promotes physiological and pathological wound healing. Arterioscler Thromb Vasc Biol 2009;29:503-10.
73. Hong SJ, Jia SX, Xie P, et al. Topically delivered adipose derived stem cells show an activated-fibroblast phenotype and enhance granulation tissue formation in skin wounds. PLoS One 2013;8:e55640.
74. Rigotti G, Marchi A, Galie M, et al. Clinical treatment of radiotherapy tissue damage by lipoaspirate transplant: a healing process mediated by adipose-derived adult stem cells. Plast Reconstr Surg 2007;119:1409-22.
75. Altman AM, Yan Y, Matthias N, et al. IFATS collection: Human adipose-derived stem cells seeded on a silk fibroin-chitosan scaffold enhance wound repair in a murine soft tissue injury model. Stem Cells 2009;27:250-8.
76. Lee HC, An SG, Lee HW, et al. Safety and effect of adipose tissue-derived stem cell implantation in patients with critical limb ischemia: a pilot study. Circ J 2012;76:1750-60.
77. Amann B, Ludemann C, Ratei R, et al. [Autologous bone-marrow stem-cell transplantation for induction of arteriogenesis for limb salvage in critical limb ischaemia]. Zentralbl Chir 2009;134:298-304.
78. Tateishi-Yuyama E, Matsubara H, Murohara T, et al. Therapeutic angiogenesis for patients with limb ischaemia by autologous transplantation of bone-marrow cells: a pilot study and a randomised controlled trial. Lancet 2002;360:427-35.
79. Esato K, Hamano K, Li TS, et al. Neovascularization induced by autologous bone marrow cell implantation in peripheral arterial disease. Cell Transplant 2002;11:747-52.
80. Miyamoto K, Nishigami K, Nagaya N, et al. Unblinded pilot study of autologous transplantation of bone marrow mononuclear cells in patients with thromboangiitis obliterans. Circulation 2006;114:2679-84.
81. Idei N, Soga J, Hata T, et al. Autologous bone-marrow mononuclear cell implantation reduces long-term major amputation risk in patients with critical limb ischemia: a comparison of atherosclerotic peripheral arterial disease and Buerger disease. Circ Cardiovasc Interv 2011;4:15-25.
82. Eming SA, Krieg T, Davidson JM. Inflammation in wound repair: molecular and cellular mechanisms. J Invest Dermatol 2007;127:514-25.
83. Gonzalez MA, Gonzalez-Rey E, Rico L, et al. Adipose-derived mesenchymal stem cells alleviate experimental colitis by inhibiting inflammatory and autoimmune responses. Gastroenterology 2009;136:978-89.
84. Pinheiro CH, de Queiroz JC, Guimaraes-Ferreira L, et al. Local injections of adipose-derived mesenchymal stem cells modulate inflammation and increase angiogenesis ameliorating the dystrophic phenotype in dystrophin-deficient skeletal muscle. Stem Cell Rev 2012;8:363-74.
85. Yun IS, Jeon YR, Lee WJ, et al. Effect of human adipose derived stem cells on scar formation and remodeling in a pig model: a pilot study. Dermatol Surg 2012;38:1678-88.
86. Park BS, Jang KA, Sung JH, et al. Adipose-derived stem cells and their secretory factors as a promising therapy for skin aging. Dermatol Surg 2008;34:1323-6.
87. Kim JH, Jung M, Kim HS, et al. Adipose-derived stem cells as a new therapeutic modality for ageing skin. Exp Dermatol 2011;20:383-7.
88. Kim WS, Park BS, Park SH, et al. Antiwrinkle effect of adipose-derived stem cell: activation of dermal fibroblast by secretory factors. J Dermatol Sci 2009;53:96-102.
89. Kim WS, Park BS, Sung JH. Protective role of adipose-derived stem cells and their soluble factors in photoaging. Arch Dermatol Res 2009;301:329-36.
90. Murakami T, Fujimoto Y, Yasunaga Y, et al. Transplanted neuronal progenitor cells in a peripheral nerve gap promote nerve repair. Brain Res 2003;974:17-24.
91. Cui L, Jiang J, Wei L, et al. Transplantation of embryonic stem cells improves nerve repair and functional recovery after severe sciatic nerve axotomy in rats. Stem Cells 2008;26:1356-65.
92. Mantovani C, Terenghi G, Shawcross SG. Isolation of adult stem cells and their differentiation to Schwann cells. Methods Mol Biol 2012;916:47-57.
93. Shen CC, Yang YC, Liu BS. Peripheral nerve repair of transplanted undifferentiated adipose tissue-derived stem cells in a biodegradable reinforced nerve conduit. J Biomed Mater Res A 2012;100:48-63.
94. Mohammadi R, Azizi S, Delirezh N, et al. Comparison of beneficial effects of undifferentiated cultured bone marrow stromal cells and omental adipose-derived nucleated cell fractions on sciatic nerve regeneration. Muscle Nerve 2011;43:157-63.
95. Amoh Y, Li L, Katsuoka K, et al. Multipotent nestin-positive, keratin-negative hair-follicle bulge stem cells can form neurons. Proc Natl Acad Sci U S A 2005;102:5530-4.
96. Amoh Y, Li L, Campillo R, et al. Implanted hair follicle stem cells form Schwann cells that support repair of severed peripheral nerves. Proc Natl Acad Sci U S A 2005;102:17734-8.
97. Walsh SK, Gordon T, Addas BM, et al. Skin-derived precur-

sor cells enhance peripheral nerve regeneration following chronic denervation. Exp Neurol 2010;223:221-8.
98. Marconi S, Castiglione G, Turano E, et al. Human adipose-derived mesenchymal stem cells systemically injected promote peripheral nerve regeneration in the mouse model of sciatic crush. Tissue Eng Part A 2012;18:1264-72.
99. Lopatina T, Kalinina N, Karagyaur M, et al. Adipose-derived stem cells stimulate regeneration of peripheral nerves: BDNF secreted by these cells promotes nerve healing and axon growth de novo. PLoS One 2011;6:e17899.
100. Lattanzi W, Geloso MC, Saulnier N, et al. Neurotrophic features of human adipose tissue-derived stromal cells: *in vitro* and *in vivo* studies. J Biomed Biotechnol 2011; 2011:468705.