molecular pharmaceutics

REVIEW

pubs.acs.org/molecularpharmaceutics

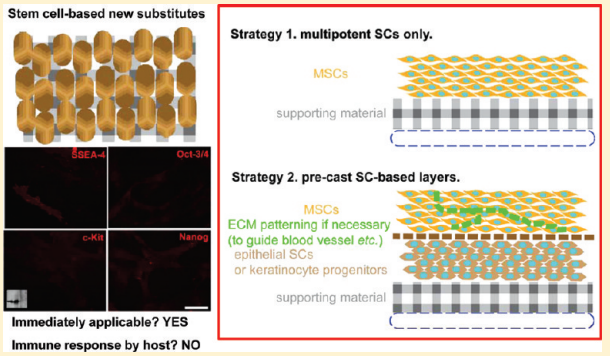
Combined Gene and Stem Cell Therapy for Cutaneous Wound Healing

Gerd G. Gauglitz[†] and Marc G. Jeschke[*,‡]

[†]Department of Dermatology and Allergy, Ludwig Maximilian University, Munich, Germany

[‡]Sunnybrook Health Sciences Centre, Department of Surgery, Division of Plastic Surgery, University of Toronto, Canada

ABSTRACT: In current medical practice, wound therapy remains a clinical challenge and much effort has been focused on the development of novel therapeutic approaches for wound treatment. Gene therapy, initially developed for treatment of congenital defects, represents a promising option for enhancing wound repair. In order to accelerate wound closure, genes encoding for growth factors or cytokines have shown the most potential. The majority of gene delivery systems are based on viral transfection, naked DNA application, high pressure injection, and liposomal vectors. Besides advances stemming from breakthroughs in recombinant growth factors and bioengineered skin, there has been a significant increase in the understanding of stem cell biology in the field of cutaneous wound healing. A variety of sources, such as bone marrow, umbilical cord blood, adipose tissue and skin/hair follicles, have been utilized to isolate stem cells and to modulate the healing response of acute and chronic wounds. Recent data have demonstrated the feasibility of autologous adult stem cell therapy in cutaneous repair and regeneration. Very recently, stem cell based skin engineering in conjunction with gene recombination, in which the stem cells act as both the seed cells and the vehicle for gene delivery to the wound site, represents the most attractive field for generating a regenerative strategy for wound therapy. The aim of this article is to discuss the use and the potential of these novel technologies in order to improve wound healing capacities.



KEYWORDS: stem cells, adult, embryonic, wound healing, umbilical cord, gene therapy

INTRODUCTION

World-wide millions of patients suffer from acute and chronic wounds due to infections, trauma, or underlying medical conditions. Particularly chronic wounds severely affect the patient's quality of life and generate enormous medical costs.[1,2] Skin acts as an essential barrier, protecting organisms from their environment. Loss of the integrity of large portions of the skin as a result of injury may lead to major disability or even death. Thus, appropriate wound care is critical, and various treatment modalities have been utilized to improve the wound bed. The overall therapy for nonhealing wounds has mainly focused on the identification and correction of the precipitating and perpetuating factors.[3] This approach includes antibiotic use for accompanying cellulitis, revascularization of ischemic limbs, rigorous off-loading for decubitus (pressure) ulcers and compression devices for venous ulcers.[3] In the past decades, increasing advancement in understanding the molecular and cellular mechanisms underlying wound repair and regeneration has led to extensive usage of growth factor supplements in wound care.[4−7,5,8−11] Growth factors and cytokines play major roles in the well-orchestrated integration of the complex biological and molecular events underlying cutaneous wound healing, including cell migration and proliferation, extracellular matrix deposition, angiogenesis, and remodeling. However, the clinical effects of the topical application of single growth factors to accelerate wound healing have been discouraging: on the one hand due to the complexity of the wound healing cascade and on the other due to the combined effects of physical inhibition and biological degradation leading to inherent loss of drug activity of the topically applied cytokines. The development of gene transfer technology has been shown to be a promising means to overcome the limitations associated with the (topical) application of recombinant proteins by delivering the respective growth factor directly to the wound bed. Stem cells, due to their pluripotency and their growth potential, make them a potentially useful vehicle for gene delivery to injury site. Thus, several novel technologies are under active development to aid cutaneous wound repair in order to enhance and accelerate the treatment of acute and chronic wounds, which are discussed in this article.

GENE THERAPY

Gene therapy, defined as the insertion of a gene into recipient cells, was initially considered only as a treatment option for patients with a congenital defect of a metabolic function or a late-stage malignancy.[12] However, possibilities of using the skin for

Special Issue: Emerging Trends in Gene- and Stem-Based Combination Therapy

Received: March 21, 2011
Accepted: June 9, 2011
Revised: May 30, 2011
Published: June 09, 2011

somatic gene therapy have been investigated now for more than 20 years.[13] Two basic strategies for introduction and expression of foreign DNA into host cells are

1. gene therapy, which is based on the permanent insertion of DNA, and
2. gene medicine, which is used for transient transformation and short-term expression of a gene product.[14]

Furthermore, genes can be delivered by either *in vivo* or *ex vivo* approaches. *In vivo* techniques are based upon the direct introduction of genes to the target tissue. *Ex vivo* techniques rely upon the isolation and cultivation of selected cells with their transfection *in vitro* and a subsequent transplantation to a host. In both approaches, the selection of an appropriate vector for the introduction of genes is paramount for success.[15]

Gene transfer with viral vectors relies on the ability of viruses to carry and express their genes into host cells. Viruses are the vectors providing high transfection efficiency and a rapid transcription of the foreign material inserted to the viral genome. However, the production of viral vectors is time and cost consuming, transfection efficacy is very variable, and the risk of local or systemic infections, leading to fatal outcomes, remains a large concern. Thus, many clinical trials in which viral vectors are being used have been interrupted since the application of these vectors induced unexpected adverse effects.[16]

Nonviral gene therapy in contrast has the advantage that gene transfer is performed without a viral vector, which eliminates the risk of infection and cost of vector production.[17] Especially the use of cationic lipid (CL) formulations for the delivery of genes appears to be safe, efficacious, and clinically applicable. On the negative side, some nonviral gene transfer methods tend to be nonspecific, and a high variability in the level of gene expression has been reported.

Genetic material—in the form of both viral and nonviral vectors—can be transferred directly to the skin. Naked DNA applied topically onto the skin leads to gene expression, but the expression is transient and at low levels.[18] Plasmid DNA in various liposomal spray formulations sprayed onto mouse or human skin likewise leads to gene expression, but at low levels.[19] A novel gene transfer method based on biphasic lipid vesicles is reported to enhance DNA transfer through intact stratum corneum.[20] Higher levels of gene expression after direct delivery can be obtained using intracutaneous injection of DNA, liposomes, a gene gun or electroporation.[13,21] Hengge et al. first developed the direct injection of DNA coding for interleukin-8 genes by injecting naked genes into the skin and obtained a significant recruitment of dermal neutrophils.[22] Eriksson and group modified the direct injection technique and developed "microseeding", delivering naked DNA into target cells via solid needles.[23] Gene gun is a technique used to penetrate the cellular membrane with which the gold or tungsten-coated particles carrying DNA plasmids are propelled into skin cells.[24] Newly developed electroporation is effective with nearly all cells and even the intact tissues. However, the disadvantages accompanying this technology include cell damage when the pulses are of the wrong length or intensity and the possibility that transport of material into and out of cells nonspecifically could later lead to improper cell functions.[25]

The use of living cells as a gene delivery vehicle has thus caused more and more attention for gene therapy. The molecular genetic approach introduces plasmid DNA or genes encoding for certain signaling factors into the cells that act as gene delivery vehicles. Required functional mediators can be expressed and secreted spatially and temporally by the transfected cells. The cellular based gene delivery is advantageous in that cells can be manipulated much more precisely than in the body. For wound therapy, these cells for genetic engineering are mostly selected on the basis of their availability, expansion capacity *in vitro*, survivability after transplantation, and differentiation properties at the injury site.[26]

Gene Therapy and Wound Healing. Several strategies have been investigated with the purpose of promoting wound healing by the use of gene transfer, including stimulation of the granulation process, the vascularization, the re-epithelialization or the scar quality.[27,28]

A potential problem of single growth factor gene therapy is that increasing the concentration of a single growth factor may not promote all phases of wound healing. A single growth factor cannot counteract all the deficiencies of a burn wound, nor can it control the complexities of chronic wound healing. Thus, recent progress in this field includes gene transfer of multiple genes to improve the wound healing process.[29,30]

Lynch et al.[31] demonstrated that the combination of PDGF and IGF-I was more effective than either growth factor alone in a partial thickness wound healing model created with the use of a dermatome. Sprugel et al.[15] found that a combination of PDGF and FGF-2 increased the DNA content of wounds in the rat better than any single growth factor. Our group subsequently investigated the feasibility of multiple cDNA constructs, using multiple genes (KGF and IGF-I cDNA), and compared it to the administration of the same genes individually.[30] Accelerated re-epithelialization, increased proliferation, and decreased skin cell apoptosis were noted. The re-epithelialization in the burn model was over twice that of the untreated control with a significant improvement in cell survival.[30] Transfection of multiple growth factor genes at strategic time points of wound healing (known as sequential growth factor therapy) is therefore the next logical step in augmenting wound healing. Other delivery routes such as biomaterials,[32] calcium phosphate transfection,[33] diethylaminoethyl-dextran,[34] and microbubble-enhanced ultrasound[35] have been investigated. Slow-release matrices[36] and gene-delivering gel/matrix products[37] allow for prolonged transgenic expression. The concept of a genetic switch is another exciting development, where transgenic expression in target cells can be switched "on" or "off", depending on the presence of or absence of a stimulator such as tetracycline.[38] Biotechnological refinements, such as wound chamber technique,[39] may also improve the efficacy of gene delivery to wounds. These new techniques show promise, but need further studies to define the efficacy and clinical applicability. More studies are also needed to define growth factor levels in different phases of wound healing and to elucidate the precise timing of gene expression or downregulation required to better augment wound healing and control of scar formation.

STEM CELLS

Stem cells, defined on the basis of the findings of Ernest A. McCulloch and James E. Till,[40,41] are characterized by their prolonged self-renewal capacity and by their asymmetric replication.[3,42] Asymmetric replication describes a special property of stem cells: with every cell division, one of the cells retains its self-renewing capacity, whereas the other enters a differentiation pathway and joins a mature nondividing population.[42] In 1981, pluripotent cells were found in the inner cell mass of the

mouse embryo, and the term "embryonic stem (ES) cell" was coined.

ES cells are pluripotent stem cells that are harvested from the inner cell mass of the preimplantation blastocyst (3−5-day-old embryo), and have been obtained from mice, nonhuman primates, and humans.[43,44] ES cells can be maintained in culture as undifferentiated cell lines or induced to differentiate into many different lineages, including blood cells, neural cells, adipocytes, muscle cells, and chondrocytes, among others.[45,46] Despite their unique potential, the use of embryonic stem cells has remained controversial. Opponents of ES cell use most often question the morality of utilizing cells obtained from destroyed embryos, an act they consider equivalent to destroying human life.[47] Current research has thus been directed toward methods to produce human ES cells without destroying embryos which gleaned promising results.[48−50] Nevertheless, the clinical application of ES cells may be limited since they represent an allogenic resource and thus have the potential to evoke an immune response.[51] In recent years, differentiated adult tissues have been shown to harbor pluripotent stem cells with unexpected plasticity[45] avoiding any immune rejection complications.

■ ADULT STEM (AS) CELLS AND CUTANEOUS WOUNDHEALING

AS cells are, especially in the area of hematopoietic stem cells, better understood than any other aspect of stem cell biology.[52] Adult stem cells tend to be tissue specific, self-renewing populations of cells which can differentiate into cell types associated with the organ system in which they reside.[43,53] It is known that niches of stem cells exist in many tissues, such as bone marrow, peripheral blood, brain, spinal cord, skeletal muscle, and epithelia of the skin and digestive system, as well as pancreas and dental pulp.[54−56] Of these, the most widely studied are CD34+ hematopoietic stem cells isolated from bone marrow. These cells are capable of producing cells of the lymphoid and myeloid lineages in blood. CD34+ cells are the only currently available therapeutic application of stem cells and are used for a variety of purposes.[51] A notable exception to the tissue specificity of adult stem cells is the bone marrow derived mesenchymal stem cell, or what is more recently called the multipotent adult progenitor cell.[51,55] A single bone marrow-derived stem cell is able to differentiate into epithelial cells of the liver, lung, gastrointestinal tract, and skin.[57,58] It has been shown that long-term repopulation by green fluorescent protein-labeled bone marrow-derived cell transplantation in wounded skin results in differentiation into nonhematopoietic skin structures.[52] Alternative sources of adult stem cells in the context of cutaneous wound healing currently discussed in this field include umbilical cord, adipose tissue and the skin itself.

Bone Marrow-Derived Stem Cells. These cells are typically obtained from bone marrow aspirates from marrow transplant donors. In addition to their ability to differentiate into multiple cell lineages, the use of marrow stem cells is advantageous because they offer a source of cells that is isolated and expanded *in vitro* with relative ease. The number of cells may be significantly increased by subculturing a small sample of donor tissue.[59,60]

Bone marrow-derived cells utilized to support healing of chronic wounds have been of particular interest. Fathke et al.[61] showed in the chimeric mouse model that distant bone marrow-derived cells may contribute to the reconstitution of the dermal fibroblast population in cutaneous wounds. Bone marrow stroma cells were further found to synthesize higher amounts of collagen, FGF, and VEGF, when compared to native dermal fibroblasts, indicating a potential benefit for accelerating wound healing.[62] Ichoka and group[63] investigated the effect of a bone marrow impregnated collagen matrix on wound healing in a microcirculatory mouse model and observed significant increases in angiogenesis. Badiavas and Falanga published clinical results using autologous bone marrow cells directly applied on chronic cutaneous ulcerations in three patients with wounds resistant to standard conventional treatment for more than 1 year. All patients showed enhancement of their wounds within days following administration, characterized by an overall decrease in wound size and an increase in the vascularity of the dermis and the dermal thickness of the wound bed.[64] Falanga et al.[65] successfully utilized a fibrin polymer spray to apply cultured autologous mesenchymal stem cells obtained from bone marrow aspirates to wounds. The technique accelerated the rate of healing of acute and nonhealing cutaneous wounds in both humans and mice. This approach may represent a practicable method for introducing cells into wounds although some difficulties are related to these techniques. Stem cells must be cultured in sufficient numbers for topical application and have to grow in the wound for a therapeutic response. In severe burn trauma, bone marrow suppression has been observed and limits their use.[66,67] Also, the number of bone marrow mesenchymal stem cells significantly decreases with age.[68]

Umbilical Cord (Blood) Derived Stem Cells. Human umbilical cord blood is a rich source of hemopoietic stem cells for clinical application.[69,70] McGuckin et al.[71] proposed umbilical cord blood to be one of the largest untouched sources of stem cells with characteristic features such as naive immune status. However, the presence of mesenchymal stem cells in umbilical cord blood is controversially discussed. Studies by Erices et al.[72] showed that mesenchymal stem cells together with hematopoietic precursors are circulating in the blood of preterm fetuses, while Mareschi et al.[73] failed to isolate these cells in preterm umbilical cord blood. In contrast, Romanov et al.[74] found that cord vasculature contains a large amount of mesenchymal stem cell-like elements. They suggested that the umbilical cord stroma could thus be utilized as an alternative source of mesenchymal stem cells for experimental and clinical investigation. A study by Kamolz et al.[75] indicated that stem cells from umbilical cord blood are able to differentiate into epithelial cells under *in vitro* conditions and suggested their use as a starting material for isolation and expansion of cells in large skin defects. However, it should be kept in mind that umbilical cord blood-derived stem cells are usually obtained from allogenic sources, potentially leading to immunological rejection upon transplant or transfusion.

Phan and colleagues recently found the amniotic membrane of the umbilical cord to be an extremely rich source of stem cells for burn resurfacing (unpublished data). The isolated cells, termed "cord lining stem cells", can be divided into subpopulations of epithelial cells (cord lining epithelial cells)[76] and mesenchymal cells (cord lining mesenchymal cells). They express stem cell markers, such as Oct-4 and Nanog, and have been shown to form healthy colonies in culture. Our group has recently shown that the amniotic membrane is a source of mesenchymal stem cells that can differentiate into bone, cartilage, and fat (Figures 1 and 2).[76] Both have been successfully utilized to treat partial thickness and full thickness burns as well as chronic diabetic wounds in an ongoing clinical case series (unpublished data). Growing these cells on specific scaffolds creates an accessible way of applying these cells to the wound. Immunological rejection of these cells

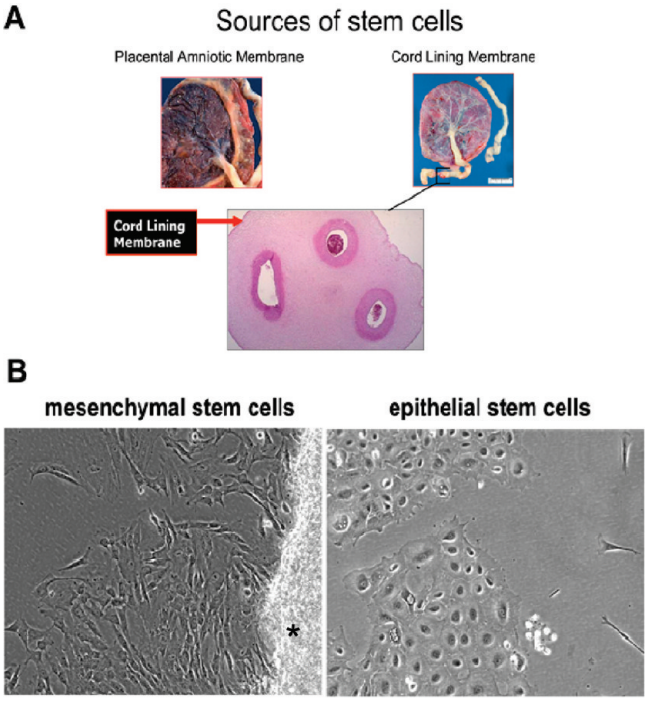


Figure 1. Overview of isolation of cells. (A) Pictures of amniotic membrane (left) and umbilical cord (right). The bottom picture shows the dissected umbilical cord. (B) Phase contrast microscopic images of cells. Left image shows typically observed mesenchymal cells. Asterisk indicates a part of implant. Right image shows a minor population of epithelial-like cells. Reprinted with permission from ref 76. Copyright 2009 Mary Ann Liebert, Inc.

has not been observed in ongoing trials nor did these cells show any anchorage-independent growth in soft agar, which represents a tumor cell-specific feature. In a recent study, Miki et al. also showed that epithelial SCs from amniotic membrane did not form tumors in nude mice.[77] Thus, subamniotic MSCs appear to be safe in terms of potential tumorgenicity.

Adipose-Derived Stem Cells (ADSCs). Adipose tissue has been identified as a source of multipotent cells which have characteristics similar to those of BM-MSCs[78] and have the capacity to differentiate to cells of adipogenic, chondrogenic, myogenic, and osteogenic lineages when cultured with the appropriate lineage specific stimuli.[79−81] ADSCs can be obtained from the processing of either liposuctioned or excised fat. Given their convenient isolation compared with BM-MSCs and extensive proliferative capacities *ex vivo*, ADSCs hold great promise for usage in wound repair and regeneration. Human liposuction aspirates were utilized by Huang et al.[82] to culture adipo-derived mesodermal stem cells and differentiate them into chondrogenic cells. A study by Kim et al.[78] indicated that adipose-derived stem cells promoted human dermal fibroblast proliferation by direct cell-to-cell contact and by secretory induced paracrine activation which significantly accelerated the re-epithelialization of cutaneous wounds. ADSCs are limited, however, by several factors. First, ADSCs have not been classified as immortal. ADSCs display obvious signs of "old age", thus limiting their capacity for subculturing. Additionally, adipose tissue is known to vary in metabolic activity and in its capacity for proliferation and differentiation, depending on the location of the tissue depot and the age and gender of the patient.[83,84]

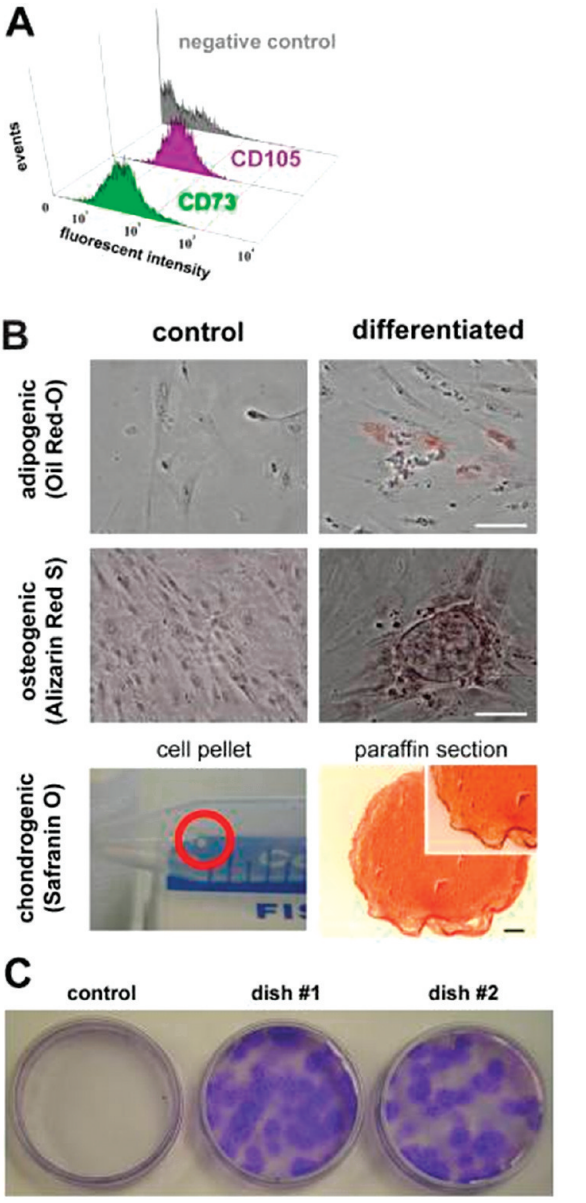


Figure 2. Characteristics of mesenchymal stem cells. (A) Expression of CD73 (green) and CD105 (purple) analyzed by flow cytometry. (B) Differentiation of mesenchymal stem cells into osteogenic, adipogenic, and chondrogenic lineages. Undifferentiated cells or differentiated cells were stained with Alizarin Red S (osteogenic), Oil-Red O (adipogenic), or Safranin O (chondrogenic). Cell pellet after chondrogenic differentiation is shown in the red circle. Scale bars = 100 μm (adipogenic and chondrogenic) and 200 μm (chondrogenic), respectively. (C) CFU-F assay: left, no cells; middle and right, dish #1 and #2. Reprinted with permission from ref 76. Copyright 2009 Mary Ann Liebert, Inc.

Epidermal Stem Cells. The skin is composed of two parts: the epidermis, the cells of which form the barrier; and the dermis, which provides support and nutrition to the epidermis. The epidermis is a multilayered epithelium that is composed of sweat glands, hair follicles (HFs) and their sebaceous glands and interfollicular epidermis.[85] The different epidermal compartments undergo constant cellular turnover to replace the dead or damaged cells. This homeostatic process is thought to involve several types of stem cells, each located in a specific epidermal

region and contributing to the maintenance of a discrete compartment of the skin.[86] In addition to their self-renewing capacity and multipotency, these cells are quiescent with a low tendency to divide, but upon injury they are characterized by an extensive and sustained self-renewal capacity.[87]

The first evidence that skin stem cells can differentiate into interfollicular epidermis, sebaceous gland and hair follicle lineages came from transplantation of bulge stem cells: a cell population located at the base of hair follicles.[88] Using transplantation of the murine bulge region, Oshima et al.[89] demonstrated that bulge cells could repopulate the epidermis, sebaceous glands, and the epithelial layers of the hair follicle. Roh et al.[90] showed that stem cells extracted from the human bulge region can be induced to exhibit hair follicle differentiation and form epidermal and sebaceous cells *in vitro* as well as form an epidermis and sebaceous cells *in vitro*, thus supporting the multipotential capacity of human epidermal stem cells. Recent work from Langton et al.[91] addressed the role of hair follicle bulge-derived stem cells and interfollicular stem cells in skin wound healing. This study utilized an animal model that entirely lacks the hair follicle bulge on the tail and trunk and does not form primary hair placodes. They observed a delay in wound re-epithelialization compared to wild-type animals, and an expanded area of interfollicular epidermis was shown to be recruited to achieve closure and re-formation of the epidermal barrier, suggesting an important role for both follicular and interfollicular stem cells in wound healing. Most authors agree that these will become an important source for therapeutic applications, which is also based on the easy access to skin.

■ ADULT STEM CELLS AS GENE DELIVERY VEHICLES FOR WOUND HEALING

In a renewing tissue such as epidermis, cells are continuously shed into the environment, and this represents a major complication to design a long-lasting gene therapy. In human epidermis most cells are replaced every 26−28 days, and therefore any persistent genetic defect needs to be present in the stem cells, with expression passed to daughter cells at each cell division.[92] Thus, *ex vivo* cultivation of epidermal stem cells and stable integration of the exogenous gene into the epidermal stem cell genome is required before the corrected cells can be reintroduced into the host. The long-term follow-up of patients with burns treated with autologous cultivated epidermal sheets is the best evidence that, under appropriated culture conditions, stem cells can be maintained in culture and grafted back to patients.

The stem cell based gene delivery strategy mainly includes three steps. First, stem cells are isolated and expanded *in vitro*. Second, the therapeutic gene is transduced into the stem cells, applying methods similar to those used in direct gene transfer. Finally, the genetically modified stem cells are returned to the patient. Recombinant retrovirus and lentivirus have been the most commonly utilized vehicles for gene transfer to epidermal keratinocytes and skin substitutes. Adenovirus and nonviral gene transfer technologies have also been used but to a much lesser extent.[16]

A recent study reported success in long-term human skin regeneration from a single genetically modified stem cell. Here, human keratinocytes were transduced by retroviral or lentiviral GFP vectors. Holoclones were then selected by their high clonogenicity, growth rate and cell number/area ratio characteristic. Skin equivalents generated from these holoclones showed normal epidermal architecture similar to that of native human skin,[93] suggesting that this may be a useful approach for tissue engineered skin. Mathor et al.[94] utilized clonogenic keratinocytes with the characteristics of cutaneous stem cells. The authors were able to induce interleukin-6 (IL-6) cDNA using a retroviral construct to achieve a stable transgene expression *in vitro*. Epidermal sheets from these transfected keratinocytes grafted onto athymic animals demonstrated IL-6 production for up to seven days after grafting. *In vivo* approaches include a recent study by Hachiya et al.[95] in which different lentiviral vectors were utilized to transfect human skin grafted onto severe combined immunodeficient mice. These mice showed an effective targeting of epidermal stem cells which underwent terminal differentiation resulting in transgene expression. In another study, transcriptionally targeted lentiviral vectors efficiently transduced clonogenic stem/progenitor cells derived from a skin biopsy of a junctional epidermolysis bullosa patient. They restored normal synthesis of laminin-5 in cultured keratinocytes, and reconstituted normal adhesion properties in human skin equivalents transplanted onto immunodeficient mice.[96]

Retroviral vectors encoding reporter genes have also been utilized to transduce cultured hair follicle stem cells. After skin reconstitution, transgene expression was observed in all skin epithelial compartments including the hair follicle epithelium, sebaceous gland, and epidermis. In addition, transgene expression was observed for at least 6 months.[97] Our group has shown that nonviral cationic liposomes carrying genes encoding for β-galactosidase were able to transfect hair follicle cells at the edge of the wound.[98] This was confirmed by positive immunohistochemical staining in epithelial stem cells both on the outer root sheath and on the matrix of hair follicles.

Currently, a system that combines nonviral gene carrier and the three-dimensional scaffold in which the stem cells are transfected with the genes released from or substrated to a 3D polymer scaffold may represent the most attractive strategy for stem cell recombination for wound therapy.[99] By using 3D scaffolds a larger surface area for cell attachment, spreading, proliferation and adhesion than in two-dimensional culture plates is provided and an environment with persisting release of target genes for the multiple transfection of cells is available. Additionally, the 3D scaffold surface controls the spatial arrangement of cells and their transmission of biochemical and mechanical signals that govern gene expression.[100] Using this approach, genetic recombinant stem cells actually act both as seed cells for tissue engineering and as the vehicle for gene delivery needed for the wound repair and regeneration.[101] Luu et al. reported the potential of a silk fibroin-chitosan (SFCS) scaffold serving as a delivery vehicle for human ADSCs in a murine soft tissue injury model. Green fluorescent protein (GFP)-labeled ADSCs were seeded on SFCS scaffolds for 48 h and then transplanted to full thickness skin defect in mice. It was shown that the extent of wound closure was significantly enhanced in the ADSC-SFCS group compared to the SFCS and no-graft control groups. Microvessel density at wound bed biopsy sites was significantly higher in the ASC-SFCS group compared to SFCS alone.[102]

■ CONCLUSIONS

In spite of recent advances from breakthroughs in recombinant growth factors, gene therapy and bioengineered skin, up to 50% of chronic wounds that have been present for more than a year remain resistant to successful treatment. Gene therapy has evolved from a purely experimental scientific endeavor in a clinically

applicable method with countless target organ systems. In wound healing, there still remain challenges such as the identification of optimal target cells, development of sequential therapy methods, and identification of factors detrimental to the introduction of genes into the wound. Stem cells, due to their ability to differentiate into various tissue types by asymmetric replication, thus represent a promising tissue repair strategy. A variety of sources have been utilized to isolate stem cells in order to modulate the healing response of acute and chronic wounds. Recent data have demonstrated the feasibility of autologous ASC therapy in cutaneous repair and regeneration. Taking into account the main qualities of the therapeutic options discussed, the transfection of cutaneous stem cells with an optimized gene therapy vehicle could provide a promising future approach. Tissue-engineered skin composed of genetically modified stem cells to promote healing has been also shown promising results. The use of stem cells as a biological basis for tissue engineering coupled with advance in gene delivery and biomaterials thus provides a promising alternative for the repair, replacement and regeneration of damaged tissues. However, translating the biological properties and potential of stem cells into therapies will require overcoming significant cell-manufacturing and regulatory challenges. Bioprocess engineering fundamentals, including bioreactor design and process control, need to be combined with cellular systems biology principles to guide the development of next-generation technologies capable of producing cell-based products in a safe, robust, and cost-effective manner. The costs accrued to date for allografts amount to approximately 1000 $ per ft^2, for amnion to 1500 to 2000 $. Other skin substitutes including CEA, Integra and CSS may be even more expensive. The use of stem cells (e.g., ADSCs, cord lining mesenchymal stem cells) as an alternative source for currently available skin substitutes may thus represent an economically reasonable approach in the future with a potential high impact in third world countries. Nevertheless, even though improvement in wound healing has been demonstrated in many stem cell treatment reports, various issues have to be considered when applying stem cells to wound patients. Particularly the age of patients is of major importance, since the functionality of stem cells in older patients seems to decrease with age, although the quantity does not seem to be affected.[103,104] In a mouse model, it has even been shown that BM-MSC from old mice inhibit rather than promote wound healing when applied to wounds in diabetic mice.[105] Also, since aspiration of MSC from the BM represents an invasive procedure itself, the use of autologous ASC for treating older patients may be less suitable than applying allogeneic ASC. In this context, immune tolerance-promoting strategies need to be investigated in the future and the mode of stem cell delivery still represents a challenging question (e.g., acellular dermal matrix as carrier for delivering ADSC to the wounds locally vs injection of stem cells), ultimately determining the success of stem cell therapy.

Despite their potential, the use of stem cells in general is critically debated. However, in fact, plastic surgeons and burns surgeons have been using stem cell therapies for wound care for well over one hundred years. Skin grafts, of necessity, have to contain stem cells or they will not survive in the long term. When Rheinwald and Green started keratinocyte culture in their laboratory, these cultures had to contain stem cells and were initially delivered in a construct containing many terminally differentiating cells. With the more recent addition of the cultured cell suspension it is probable that the cell mix contains even more stem cells. Nevertheless, whether human stem cells are of embryonic, fetal or adult origin, donor sources must be carefully screened to prevent transmission of infectious diseases, but also to assess the genetic background to obtain optimal conditions for allogenic transplantation and for particular clinical situations. To ensure the integrity, uniformity, and reliability of human stem cells, rigorously controlled and standardized procedures have to be followed in establishing and maintaining stem cell lines in culture to avoid unintended alterations in intrinsic properties.[106] Thus, further research and characterization in order to solve the many questions on their experimental and clinical applications is necessary. Useful parameters would include (1) cell morphology; (2) cell-surface marker expression; (3) tissue-specific enzymatic activity; and (4) characteristic gene expression patterns.[106]

AUTHOR INFORMATION

Corresponding Author

*Sunnybrook Health Sciences Centre, Department of Surgery, Division of Plastic Surgery, University of Toronto, Rm D704, 2075 Bayview Ave., Toronto, ON Canada M4N 3M5. Tel: 416-480-6703. Fax: 416-480-6763. E-mail: marc.jeschke@sunnybrook.ca.

ACKNOWLEDGMENT

The authors would like thank Dr. Katsu Kita, PhD, for his support and help.

ABBREVIATIONS USED

ADSCs, adipose-derived stem cells; HSCs, hematopoietic stem cell; ES cells, embryonic stem cells; AS cells, adult stem cells; MSC, mesenchymal stem cells; BM, bone marrow; MEFs, mouse embryonic fibroblasts; IPS, induced pluripotent state; FGF, fibroblast growth factor; VEGF, vascular endothelial growth factor; HDF, human dermal fibroblasts; PDGF, platelet derived growth factor; IGF, insulin-like growth factor; KGF, keratinocyte growth factor; TGF, transforming growth factor; HFs, hair follicles; CXCL5, C-X-C motif chemokine 5

REFERENCES

(1) Harrington, C.; Zagari, M. J.; Corea, J.; Klitenic, J. A cost analysis of diabetic lower-extremity ulcers. *Diabetes Care* **2000**, *23* (9), 1333–8.

(2) Boulton, A. J.; Vileikyte, L.; Ragnarson-Tennvall, G.; Apelqvist, J. The global burden of diabetic foot disease. *Lancet* **2005**, *366* (9498), 1719–24.

(3) Cha, J.; Falanga, V. Stem cells in cutaneous wound healing. *Clin. Dermatol.* **2007**, *25* (1), 73–8.

(4) Blome-Eberwein, S.; Jester, A.; Kuentscher, M.; Raff, T.; Germann, G.; Pelzer, M. Clinical practice of glycerol preserved allograft skin coverage. *Burns* **2002**, *28* (Suppl. 1), S10–12.

(5) Garfein, E. S.; Orgill, D. P.; Pribaz, J. J. Clinical applications of tissue engineered constructs. *Clin. Plast. Surg.* **2003**, *30* (4), 485–98.

(6) Munster, A. M. Cultured skin for massive burns. A prospective, controlled trial. *Ann. Surg.* **1996**, *224* (3), 372–5, discussion 375−7.

(7) Barret, J. P.; Wolf, S. E.; Desai, M. H.; Herndon, D. N. Cost-efficacy of cultured epidermal autografts in massive pediatric burns. *Ann. Surg.* **2000**, *231* (6), 869–76.

(8) Bannasch, H.; Fohn, M.; Unterberg, T.; Bach, A. D.; Weyand, B.; Stark, G. B. Skin tissue engineering. *Clin. Plast. Surg.* **2003**, *30* (4), 573–9.

(9) Pellegrini, G.; Ranno, R.; Stracuzzi, G.; Bondanza, S.; Guerra, L.; Zambruno, G.; Micali, G.; De Luca, M. The control of epidermal stem cells (holoclones) in the treatment of massive full-thickness burns with autologous keratinocytes cultured on fibrin. *Transplantation* **1999**, *68* (6), 868–79.

(10) Hohlfeld, J.; de Buys Roessingh, A.; Hirt-Burri, N.; Chaubert, P.; Gerber, S.; Scaletta, C.; Hohlfeld, P.; Applegate, L. A. Tissue engineered fetal skin constructs for paediatric burns. *Lancet* **2005**, *366* (9488), 840–2.
(11) Muller, M.; Gahankari, D.; Herndon, D. N. Operative Wound Management. In *Total Burn Care*, 3rd ed.; Herndon, D. N., Ed.; Saunders: New York, 2007; pp 177−95.
(12) Hernandez, A.; Evers, B. M. Functional genomics: clinical effect and the evolving role of the surgeon. *Arch. Surg.* **1999**, *134* (11), 1209–15.
(13) Jensen, T. G. Cutaneous gene therapy. *Ann. Med.* **2007**, *39* (2), 108–15.
(14) Khavari, P. A.; Rollman, O.; Vahlquist, A. Cutaneous gene transfer for skin and systemic diseases. *J. Intern. Med.* **2002**, *252* (1), 1–10.
(15) Sprugel, K. H.; McPherson, J. M.; Clowes, A. W.; Ross, R. Effects of growth factors in vivo. I. Cell ingrowth into porous subcutaneous chambers. *Am. J. Pathol.* **1987**, *129* (3), 601–13.
(16) Escamez, M. J.; Carretero, M.; Garcia, M.; Martinez-Santamaria, L.; Mirones, I.; Duarte, B.; Holguin, A.; Garcia, E.; Garcia, V.; Meana, A.; Jorcano, J. L.; Larcher, F.; Del Rio, M. Assessment of optimal virus-mediated growth factor gene delivery for human cutaneous wound healing enhancement. *J. Invest. Dermatol.* **2008**, *128* (6), 1565–75.
(17) Vogel, J. C. Nonviral skin gene therapy. *Hum. Gene Ther.* **2000**, *11* (16), 2253–9.
(18) Fan, H.; Lin, Q.; Morrissey, G. R.; Khavari, P. A. Immunization via hair follicles by topical application of naked DNA to normal skin. *Nat. Biotechnol.* **1999**, *17* (9), 870–2.
(19) Meykadeh, N.; Mirmohammadsadegh, A.; Wang, Z.; Basner-Tschakarjan, E.; Hengge, U. R. Topical application of plasmid DNA to mouse and human skin. *J. Mol. Med.* **2005**, *83* (11), 897–903.
(20) Foldvari, M.; Kumar, P.; King, M.; Batta, R.; Michel, D.; Badea, I.; Wloch, M. Gene delivery into human skin in vitro using biphasic lipid vesicles. *Curr. Drug Delivery* **2006**, *3* (1), 89–93.
(21) Jensen, T. G. Gene transfer into human epidermis as an experimental model for somatic gene therapy. *Dan. Med. Bull.* **2004**, *51* (2), 155–66.
(22) Hengge, U. R.; Chan, E. F.; Foster, R. A.; Walker, P. S.; Vogel, J. C. Cytokine gene expression in epidermis with biological effects following injection of naked DNA. *Nat. Genet.* **1995**, *10* (2), 161–6.
(23) Eriksson, E.; Yao, F.; Svensjo, T.; Winkler, T.; Slama, J.; Macklin, M. D.; Andree, C.; McGregor, M.; Hinshaw, V.; Swain, W. F. In vivo gene transfer to skin and wound by microseeding. *J. Surg. Res.* **1998**, *78* (2), 85–91.
(24) Johnston, S. A.; Tang, D. C. Gene gun transfection of animal cells and genetic immunization. *Methods Cell Biol.* **1994**, *43* (Part A), 353–65.
(25) Potter, H.; Heller, R. Transfection by electroporation. *Curr. Protoc. Mol. Biol.* **2010**, *92*, 9.3.1–9.3.10.
(26) Johnson-Saliba, M.; Jans, D. A. Gene therapy: optimizing DNA delivery to the nucleus. *Curr. Drug Targets* **2001**, *2* (4), 371–99.
(27) Petrie, N. C.; Vranckx, J. J.; Hoeller, D.; Yao, F.; Eriksson, E. Gene delivery of PDGF for wound healing therapy. *J. Tissue Viability* **2005**, *15* (4), 16–21.
(28) Rogers, B.; Lineaweaver, W. C. Skin wound healing and cell-mediated DNA transport. *J. Long-Term Eff. Med. Implants* **2002**, *12* (2), 125–30.
(29) Liu, P. Y.; Liu, K.; Wang, X. T.; Badiavas, E.; Rieger-Christ, K. M.; Tang, J. B.; Summerhayes, I. C. Efficacy of combination gene therapy with multiple growth factor cDNAs to enhance skin flap survival in a rat model. *DNA Cell Biol.* **2005**, *24* (11), 751–7.
(30) Jeschke, M. G.; Klein, D. Liposomal gene transfer of multiple genes is more effective than gene transfer of a single gene. *Gene Ther.* **2004**, *11* (10), 847–55.
(31) Lynch, S. E.; Nixon, J. C.; Colvin, R. B.; Antoniades, H. N. Role of platelet-derived growth factor in wound healing: synergistic effects with other growth factors. *Proc. Natl. Acad. Sci. U.S.A.* **1987**, *84* (21), 7696–700.
(32) Shea, L. D.; Smiley, E.; Bonadio, J.; Mooney, D. J. DNA delivery from polymer matrices for tissue engineering. *Nat. Biotechnol.* **1999**, *17* (6), 551–4.
(33) Fu, H.; Hu, Y.; McNelis, T.; Hollinger, J. O. A calcium phosphate-based gene delivery system. *J. Biomed. Mater. Res., Part A* **2005**, *74* (1), 40–8.
(34) Eriksson, E. Gene transfer in wound healing. *Adv. Skin Wound Care* **2000**, *13* (2 Suppl.), 20–2.
(35) Lawrie, A.; Brisken, A. F.; Francis, S. E.; Cumberland, D. C.; Crossman, D. C.; Newman, C. M. Microbubble-enhanced ultrasound for vascular gene delivery. *Gene Ther.* **2000**, *7* (23), 2023–7.
(36) Chandler, L. A.; Gu, D. L.; Ma, C.; Gonzalez, A. M.; Doukas, J.; Nguyen, T.; Pierce, G. F.; Phillips, M. L. Matrix-enabled gene transfer for cutaneous wound repair. *Wound Repair Regen* **2000**, *8* (6), 473–9.
(37) Voigt, M.; Schauer, M.; Schaefer, D. J.; Andree, C.; Horch, R.; Stark, G. B. Cultured epidermal keratinocytes on a microspherical transport system are feasible to reconstitute the epidermis in full-thickness wounds. *Tissue Eng* **1999**, *5* (6), 563–72.
(38) Gossen, M.; Bujard, H. Tight control of gene expression in mammalian cells by tetracycline-responsive promoters. *Proc Natl Acad Sci U S A* **1992**, *89* (12), 5547–51.
(39) Breuing, K.; Eriksson, E.; Liu, P.; Miller, D. R. Healing of partial thickness porcine skin wounds in a liquid environment. *J Surg Res* **1992**, *52* (1), 50–8.
(40) Becker, A. J.; Mc, C. E.; Till, J. E. Cytological demonstration of the clonal nature of spleen colonies derived from transplanted mouse marrow cells. *Nature* **1963**, *197*, 452–4.
(41) Siminovitch, L.; McCulloch, E. A.; Till, J. E. The Distribution of Colony-Forming Cells among Spleen Colonies. *J Cell Physiol* **1963**, *62*, 327–36.
(42) Weissman, I. L. Stem cells: units of development, units of regeneration, and units in evolution. *Cell* **2000**, *100* (1), 157–68.
(43) Vats, A.; Tolley, N. S.; Polak, J. M.; Buttery, L. D. Stem cells: sources and applications. *Clin Otolaryngol Allied Sci* **2002**, *27* (4), 227–32.
(44) Bishop, A. E.; Buttery, L. D.; Polak, J. M. Embryonic stem cells. *J Pathol* **2002**, *197* (4), 424–9.
(45) Hadjantonakis, A.; Papaioannou, V. The stem cells of early embryos. *Differentiation* **2001**, *68* (4−5), 159–66.
(46) Murray, P.; Edgar, D. The regulation of embryonic stem cell differentiation by leukaemia inhibitory factor (LIF). *Differentiation* **2001**, *68* (4−5), 227–34.
(47) Young, F. E. A time for restraint. *Science* **2000**, *287* (5457), 1424.
(48) Prentice, D. A. Adult stem cells. *Issues Law Med.* **2004**, *19* (3), 265–94.
(49) Chung, Y.; Klimanskaya, I.; Becker, S.; Marh, J.; Lu, S. J.; Johnson, J.; Meisner, L.; Lanza, R. Embryonic and extraembryonic stem cell lines derived from single mouse blastomeres. *Nature* **2006**, *439* (7073), 216–9.
(50) Klimanskaya, I.; Chung, Y.; Becker, S.; Lu, S. J.; Lanza, R. Human embryonic stem cell lines derived from single blastomeres. *Nature* **2006**, *444* (7118), 481–5.
(51) Hipp, J.; Atala, A. Sources of stem cells for regenerative medicine. *Stem Cell Rev.* **2008**, *4* (1), 3–11.
(52) Ballas, C. B.; Zielske, S. P.; Gerson, S. L. Adult bone marrow stem cells for cell and gene therapies: implications for greater use. *J. Cell. Biochem. Suppl.* **2002**, *38*, 20–8.
(53) Gomillion, C. T.; Burg, K. J. Stem cells and adipose tissue engineering. *Biomaterials* **2006**, *27* (36), 6052–63.
(54) Presnell, S. C.; Petersen, B.; Heidaran, M. Stem cells in adult tissues. *Semin. Cell Dev. Biol.* **2002**, *13* (5), 369–76.
(55) Spradling, A.; Drummond-Barbosa, D.; Kai, T. Stem cells find their niche. *Nature* **2001**, *414* (6859), 98–104.
(56) Jiang, Y.; Vaessen, B.; Lenvik, T.; Blackstad, M.; Reyes, M.; Verfaillie, C. M. Multipotent progenitor cells can be isolated from postnatal murine bone marrow, muscle, and brain. *Exp. Hematol.* **2002**, *30* (8), 896–904.

(57) Krause, D. S.; Theise, N. D.; Collector, M. I.; Henegariu, O.; Hwang, S.; Gardner, R.; Neutzel, S.; Sharkis, S. J. Multi-organ, multi-lineage engraftment by a single bone marrow-derived stem cell. *Cell* **2001**, *105* (3), 369–77.

(58) Badiavas, E. V.; Abedi, M.; Butmarc, J.; Falanga, V.; Quesenberry, P. Participation of bone marrow derived cells in cutaneous wound healing. *J. Cell. Physiol.* **2003**, *196* (2), 245–50.

(59) Barry, F. P.; Murphy, J. M. Mesenchymal stem cells: clinical applications and biological characterization. *Int. J. Biochem. Cell Biol.* **2004**, *36* (4), 568–84.

(60) Minguell, J. J.; Erices, A.; Conget, P. Mesenchymal stem cells. *Exp. Biol. Med. (Maywood)* **2001**, *226* (6), 507–20.

(61) Fathke, C.; Wilson, L.; Hutter, J.; Kapoor, V.; Smith, A.; Hocking, A.; Isik, F. Contribution of bone marrow-derived cells to skin: collagen deposition and wound repair. *Stem Cells* **2004**, *22* (5), 812–22.

(62) Han, S. K.; Yoon, T. H.; Lee, D. G.; Lee, M. A.; Kim, W. K. Potential of human bone marrow stromal cells to accelerate wound healing in vitro. *Ann. Plast. Surg.* **2005**, *55* (4), 414–9.

(63) Ichioka, S.; Kouraba, S.; Sekiya, N.; Ohura, N.; Nakatsuka, T. Bone marrow-impregnated collagen matrix for wound healing: experimental evaluation in a microcirculatory model of angiogenesis, and clinical experience. *Br. J. Plast. Surg.* **2005**, *58* (8), 1124–30.

(64) Badiavas, E. V.; Falanga, V. Treatment of chronic wounds with bone marrow-derived cells. *Arch. Dermatol.* **2003**, *139* (4), 510–6.

(65) Falanga, V.; Iwamoto, S.; Chartier, M.; Yufit, T.; Butmarc, J.; Kouttab, N.; Shrayer, D.; Carson, P. Autologous bone marrow-derived cultured mesenchymal stem cells delivered in a fibrin spray accelerate healing in murine and human cutaneous wounds. *Tissue Eng.* **2007**, *13* (6), 1299–312.

(66) Gamelli, R. L.; He, L. K.; Liu, H. Recombinant human granulocyte colony-stimulating factor treatment improves macrophage suppression of granulocyte and macrophage growth after burn and burn wound infection. *J. Trauma* **1995**, *39* (6), 1141–6, discussion 1146−7.

(67) Shoup, M.; Weisenberger, J. M.; Wang, J. L.; Pyle, J. M.; Gamelli, R. L.; Shankar, R. Mechanisms of neutropenia involving myeloid maturation arrest in burn sepsis. *Ann. Surg.* **1998**, *228* (1), 112–22.

(68) Rao, M. S.; Mattson, M. P. Stem cells and aging: expanding the possibilities. *Mech. Ageing Dev.* **2001**, *122* (7), 713–34.

(69) Huss, R. Isolation of primary and immortalized CD34-hematopoietic and mesenchymal stem cells from various sources. *Stem Cells* **2000**, *18* (1), 1–9.

(70) Hows, J. M. Status of umbilical cord blood transplantation in the year 2001. *J. Clin. Pathol.* **2001**, *54* (6), 428–34.

(71) McGuckin, C. P.; Forraz, N.; Baradez, M. O.; Navran, S.; Zhao, J.; Urban, R.; Tilton, R.; Denner, L. Production of stem cells with embryonic characteristics from human umbilical cord blood. *Cell Proliferation* **2005**, *38* (4), 245–55.

(72) Erices, A.; Conget, P.; Minguell, J. J. Mesenchymal progenitor cells in human umbilical cord blood. *Br. J. Haematol.* **2000**, *109* (1), 235–42.

(73) Mareschi, K.; Biasin, E.; Piacibello, W.; Aglietta, M.; Madon, E.; Fagioli, F. Isolation of human mesenchymal stem cells: bone marrow versus umbilical cord blood. *Haematologica* **2001**, *86* (10), 1099–100.

(74) Romanov, Y. A.; Svintsitskaya, V. A.; Smirnov, V. N. Searching for alternative sources of postnatal human mesenchymal stem cells: candidate MSC-like cells from umbilical cord. *Stem Cells* **2003**, *21* (1), 105–10.

(75) Kamolz, L. P.; Kolbus, A.; Wick, N.; Mazal, P. R.; Eisenbock, B.; Burjak, S.; Meissl, G. Cultured human epithelium: human umbilical cord blood stem cells differentiate into keratinocytes under in vitro conditions. *Burns* **2006**, *32* (1), 16–9.

(76) Kita, K.; Gauglitz, G. G.; Phan, T. T.; Herndon, D. N.; Jeschke, M. G. Isolation and characterization of mesenchymal stem cells from the sub-amniotic human umbilical cord lining membrane. *Stem Cells Dev.* **2010**, *19* (4), 491–502.

(77) Miki, T.; Lehmann, T.; Cai, H.; Stolz, D. B.; Strom, S. C. Stem cell characteristics of amniotic epithelial cells. *Stem Cells* **2005**, *23* (10), 1549–59.

(78) Kim, W. S.; Park, B. S.; Sung, J. H.; Yang, J. M.; Park, S. B.; Kwak, S. J.; Park, J. S. Wound healing effect of adipose-derived stem cells: a critical role of secretory factors on human dermal fibroblasts. *J. Dermatol. Sci.* **2007**, *48* (1), 15–24.

(79) Zuk, P. A.; Zhu, M.; Ashjian, P.; De Ugarte, D. A.; Huang, J. I.; Mizuno, H.; Alfonso, Z. C.; Fraser, J. K.; Benhaim, P.; Hedrick, M. H. Human adipose tissue is a source of multipotent stem cells. *Mol. Biol. Cell* **2002**, *13* (12), 4279–95.

(80) Zuk, P. A.; Zhu, M.; Mizuno, H.; Huang, J.; Futrell, J. W.; Katz, A. J.; Benhaim, P.; Lorenz, H. P.; Hedrick, M. H. Multilineage cells from human adipose tissue: implications for cell-based therapies. *Tissue Eng.* **2001**, *7* (2), 211–28.

(81) Kim, C. G.; Lee, J. J.; Jung, D. Y.; Jeon, J.; Heo, H. S.; Kang, H. C.; Shin, J. H.; Cho, Y. S.; Cha, K. J.; Do, B. R.; Kim, K. S.; Kim, H. S. Profiling of differentially expressed genes in human stem cells by cDNA microarray. *Mol. Cells* **2006**, *21* (3), 343–55.

(82) Huang, J. I.; Hedrick, M. H.; Lorenz, P. Chondrogenesis of human adipo-derived mesodermal stem cells. *Surg. Forum* **2000**, No. L, 583–585.

(83) Tholpady, S. S.; Llull, R.; Ogle, R. C.; Rubin, J. P.; Futrell, J. W.; Katz, A. J. Adipose tissue: stem cells and beyond. *Clin. Plast. Surg.* **2006**, *33* (1), 55–62, vi.

(84) Giorgino, F.; Laviola, L.; Eriksson, J. W. Regional differences of insulin action in adipose tissue: insights from in vivo and in vitro studies. *Acta Physiol. Scand.* **2005**, *183* (1), 13–30.

(85) Abbas, O.; Mahalingam, M. Epidermal stem cells: practical perspectives and potential uses. *Br. J. Dermatol.* **2009**, *161* (2), 228–36.

(86) Blanpain, C.; Fuchs, E. Epidermal homeostasis: a balancing act of stem cells in the skin. *Nat. Rev. Mol. Cell Biol.* **2009**, *10* (3), 207–17.

(87) Tiede, S.; Kloepper, J. E.; Bodo, E.; Tiwari, S.; Kruse, C.; Paus, R. Hair follicle stem cells: walking the maze. *Eur. J. Cell. Biol.* **2007**, *86* (7), 355–76.

(88) Blanpain, C. Stem cells: Skin regeneration and repair. *Nature* **2010**, *464* (7289), 686–7.

(89) Oshima, H.; Rochat, A.; Kedzia, C.; Kobayashi, K.; Barrandon, Y. Morphogenesis and renewal of hair follicles from adult multipotent stem cells. *Cell* **2001**, *104* (2), 233–45.

(90) Roh, C.; Lyle, S. Cutaneous stem cells and wound healing. *Pediatr. Res.* **2006**, *59* (4 Part 2), 100R–3R.

(91) Langton, A. K.; Herrick, S. E.; Headon, D. J. An extended epidermal response heals cutaneous wounds in the absence of a hair follicle stem cell contribution. *J. Invest. Dermatol.* **2008**, *128* (5), 1311–8.

(92) Rothberg, S.; Crounse, R. G.; Lee, J. L. Glycine-C-14-incorporation into the proteins of normal stratum corneum and the abnormal straum corneum of psoriasis. *J. Invest. Dermatol.* **1961**, *37*, 497–505.

(93) Larcher, F.; Dellambra, E.; Rico, L.; Bondanza, S.; Murillas, R.; Cattoglio, C.; Mavilio, F.; Jorcano, J. L.; Zambruno, G.; Del Rio, M. Long-term engraftment of single genetically modified human epidermal holoclones enables safety pre-assessment of cutaneous gene therapy. *Mol. Ther.* **2007**, *15* (9), 1670–6.

(94) Mathor, M. B.; Ferrari, G.; Dellambra, E.; Cilli, M.; Mavilio, F.; Cancedda, R.; De Luca, M. Clonal analysis of stably transduced human epidermal stem cells in culture. *Proc. Natl. Acad. Sci. U.S.A.* **1996**, *93* (19), 10371–6.

(95) Hachiya, A.; Sriwiriyanont, P.; Patel, A.; Saito, N.; Ohuchi, A.; Kitahara, T.; Takema, Y.; Tsuboi, R.; Boissy, R. E.; Visscher, M. O.; Wilson, J. M.; Kobinger, G. P. Gene transfer in human skin with different pseudotyped HIV-based vectors. *Gene Ther.* **2007**, *14* (8), 648–56.

(96) Di Nunzio, F.; Maruggi, G.; Ferrari, S.; Di Iorio, E.; Poletti, V.; Garcia, M.; Del Rio, M.; De Luca, M.; Larcher, F.; Pellegrini, G.; Mavilio, F. Correction of laminin-5 deficiency in human epidermal stem cells by transcriptionally targeted lentiviral vectors. *Mol. Ther.* **2008**, *16* (12), 1977–85.

(97) Sugiyama-Nakagiri, Y.; Akiyama, M.; Shimizu, H. Hair follicle stem cell-targeted gene transfer and reconstitution system. *Gene Ther.* **2006**, *13* (8), 732–7.

(98) Pereira, C. T.; Herndon, D. N.; Perez-Polo, J. R.; Burke, A. S.; Jeschke, M. G. Scar trek: follicular frontiers in skin replacement therapy. *Genet. Mol. Res.* **2007**, *6* (1), 243–249.

(99) Jo, J.; Tabata, Y. Non-viral gene transfection technologies for genetic engineering of stem cells. *Eur. J. Pharm. Biopharm.* **2008**, *68* (1), 90–104.

(100) Conrad, C.; Gupta, R.; Mohan, H.; Niess, H.; Bruns, C. J.; Kopp, R.; von Luettichau, I.; Guba, M.; Heeschen, C.; Jauch, K. W.; Huss, R.; Nelson, P. J. Genetically engineered stem cells for therapeutic gene delivery. *Curr. Gene Ther.* **2007**, *7* (4), 249–60.

(101) Zhang, H.; Lee, M. Y.; Hogg, M. G.; Dordick, J. S.; Sharfstein, S. T. Gene delivery in three-dimensional cell cultures by superparamagnetic nanoparticles. *ACS Nano* **2010**, *4* (8), 4733–43.

(102) Zhang, J.; Duan, Y.; Wei, D.; Wang, L.; Wang, H.; Gu, Z.; Kong, D. Co-electrospun fibrous scaffold-adsorbed DNA for substrate-mediated gene delivery. *J. Biomed. Mater. Res., Part A* **2010**, *96A* (1), 212–20.

(103) Chambers, S. M.; Shaw, C. A.; Gatza, C.; Fisk, C. J.; Donehower, L. A.; Goodell, M. A. Aging hematopoietic stem cells decline in function and exhibit epigenetic dysregulation. *PLoS Biol.* **2007**, *5* (8), e201.

(104) Van Zant, G.; Liang, Y. The role of stem cells in aging. *Exp. Hematol.* **2003**, *31* (8), 659–72.

(105) Schatteman, G. C.; Ma, N. Old bone marrow cells inhibit skin wound vascularization. *Stem Cells* **2006**, *24* (3), 717–21.

(106) Prelle, K.; Zink, N.; Wolf, E. Pluripotent stem cells--model of embryonic development, tool for gene targeting, and basis of cell therapy. *Anat. Histol. Embryol.* **2002**, *31* (3), 169–86.