

Gene Transfer & Stem Cells: Recent advances for treating Diabetic Foot.

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INTRODUCTION

Latest developments in molecular and cell biology help to understand and treat many of the Chronic diseases including wound healing. The different events in progress of wound healing like inflammation, proliferation and remodeling needs the coordinated and sequential activation and inactivation of gene expression programmes in response to signals from the cellular environment. There are few genetic defects that are directly linked to altered wound healing. The reverse genetic approach in recombinant mice and other species has identified many genes whose over or under expression leads, in part, to a wound healing phenotype. These studies, together with detailed, descriptive studies of patterns of gene expression in normal and abnormal healing,¹ have led to the selection of leading candidates for potential therapeutic application.

Stem cells are immature, unprogrammed cells that have the ability to grow into different kinds of tissue and can be sourced from people of all ages. The first mammalian stem cells to be studied in detail were those of the hematopoietic system, and much is understood about conditions that cause these marrow precursors to differentiate along different pathways in order to maintain appropriate concentrations of a variety of cell populations in circulating blood. Characteristically, these marrow stem cells, divide much more slowly than their surrounding, derived cell population, and they remain in a relatively undifferentiated state as long as they reside in an appropriate environment (niche).² The third type of precursor cell to be considered is the marrow-derived tissue progenitor that circulates in the bloodstream, waiting to be called into sites of injury to participate in the repair process.³

GENE TRANSFER TO WOUNDS

Many investigators had clearly established by 1990, both an intrinsic and a therapeutic role for peptide growth factors in wound healing, and many biotechnology groups had succeeded in expressing the recombinant proteins as potential therapeutic agents.⁴ However, as clinical trials with agents such as epidermal growth factor, fibroblast growth factor 2, platelet-derived growth factor, and transforming growth factor proceeded, it was quickly appreciated that very high levels of exogenous peptides would be needed in chronic, human-wounds to mimic the effects of very small amounts of similar or identical proteins expressed by the resident cells.⁵⁻⁷ Thus, several groups developed strategies to augment the putative deficiencies of peptide growth factors by introducing cDNA copies of the growth factor genes into target cells at the wound site.

HOW TO TRANSFER GENES ?

There are several potential methods, to transfer genes into skin or wounds.^{8,9} **Physical methods** involve driving the DNA vector (a purified bacterial plasmid) into tissue cells with mechanical electrical for Successful introduction of biologically active DNA into wounds has been achieved

with the "gene gun", a device that propels small, DNA-coated gold/tungsten particles into the tissue in a shotgun pattern¹⁰ a needle array that functions much like a tattooing instrument and an electrode array that uses a train of high-voltage pulses to create temporary pores in nearby cells^{11,12} **Chemical methods** of DNA delivery are less efficient but less expensive and they have included liposomes, nano particles, dried methylcellulose discs and collagen gels or scaffolds. Viruses are **natural** gene delivery systems.¹³ DNA viruses, such as adenovirus do not insert viral DNA into the host genome, and so they act as transient gene delivery-systems. Adenovirus does express proteins that can incite an inflammatory response, and newer vectors have been engineered to minimize this reaction, albeit a minor consideration for wound infection. Adeno-associated virus produces less inflammatory response, although it has limitations in the amount of genetic material it can carry and the cell types that can be infected.^{14,15} RNA viruses (retroviruses) such as Moloney sarcoma virus and the lenti viruses act by stable insertion of their genome into the host genome; thus they are more useful for gene therapy applications, in combination with a tissue engineering substitute that has a limited lifespan in the host, or by placing the gene under regulation of a drug. Traditional retroviruses infect only dividing cells, but derivatives of HIV-like lenti viruses are able to infect a wide variety of cell types.¹⁶ Transient transformation of wounds with candidate genes can result in 1-3 weeks of expression, depending on the delivery method and the choice of DNA regulatory sequence. In practice, most current protocols for wound gene transfer employ a strong, promiscuous promoter of gene expression that is derived from cytomegalovirus (CMV). Greater selectivity of gene, action can readily be achieved by using gene regulation sequences that are tissue specific or that respond to a drug/hormone such as RU-486 or tamoxifen¹⁷⁻¹⁹.

Gene transfer has achieved a successful outcome in many pre-clinical models, using cDNAs for EGF-TGF-β1, PDGF, FGF-2 vascular endothelial growth factor (VEGF) hepatocyte growth factor (HGF) and other peptides in the delivery-systems described above.⁹ A potentially attractive aspect of gene transfer is the ease of combining two genes into one DNA vector. This may be a way to develop, with a less complicated regulatory pathway, a therapy that capitalises upon the synergistic effects of growth factor or cytokine combinations.²⁰⁻²² As another strategy, gene transfer studies have also shown that (wound) cells may benefit from added expression of not only the stimulus, but the receptor for that stimulus and the machinery that transmits signals from the receptor to other cellular machinery.

Gene transfer has recently taken on a role in drug development, since it is a relatively efficient method to screen for genes that have wound-healing properties, independent of a requirement that they act on cells from the outside. Indeed, nuclear transcription factors such as HoxA3,²³ Smads 3 and 7,²⁴⁻²⁵ Egr-1,²⁶ engineered zinc finger proteins^{27,28} and cardiac ankyrin repeat protein (CARP)²⁹ as well as signal transduction molecules such as

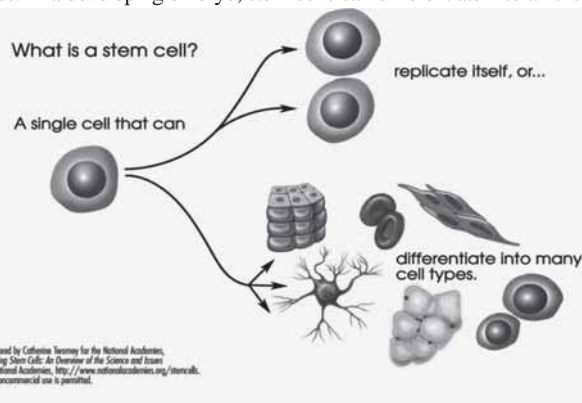
eps8, which act inside of cells are active in wounds of either normal or diabetic animal, after gene transfer. Gene transfer is thus a powerful screening method for the most effective therapeutic genes.

I would like to mention Two gene therapy clinical trials for wound healing. One is a National Institute of Health sponsored trial of PDGF-BB delivered by an adenovirus in diabetic foot ulcers³⁰. A **second**, trial completed by Tissue Repair Company, which administers adenoviral PDGF to diabetic foot ulcers. Positive findings of the latter trial were reported at the 2005 annual meeting of the Wound Healing Society. There are also many efforts to use EGF-2 and JVEGF28,31 in gene transfer experiments to improve (lower extremity) circulation. It is likely that success in these trials would have an important influence on the management and prognosis of the diabetic foot ulcer. Additional trials with FGF-2 and Vascular Endothelium Growth Factor genes or proteins for the development of collateral circulation (usually cardiovascular) may eventually have an impact on improving collateral circulation in the diabetic limb also.

STEM CELLS

In 1908 - The term "stem cell" was proposed for scientific use by the Russian histologist Alexander Maksimov (1874–1928) at congress of hematologic society in Berlin. It postulated existence of haematopoietic stem cells.

Stem cells are basic cells that can divide and differentiate into diverse specialized cell types and can self-renew to produce more stem cells. In mammals, there are two broad types of stem cells: embryonic stem cells, which are isolated from the inner cell mass of blastocysts, and adult stem cells, which are found in various tissues. In adult organisms, stem cells and progenitor cells act as a repair system for the body, replenishing adult tissues. In a developing embryo, stem cells can differentiate into all the



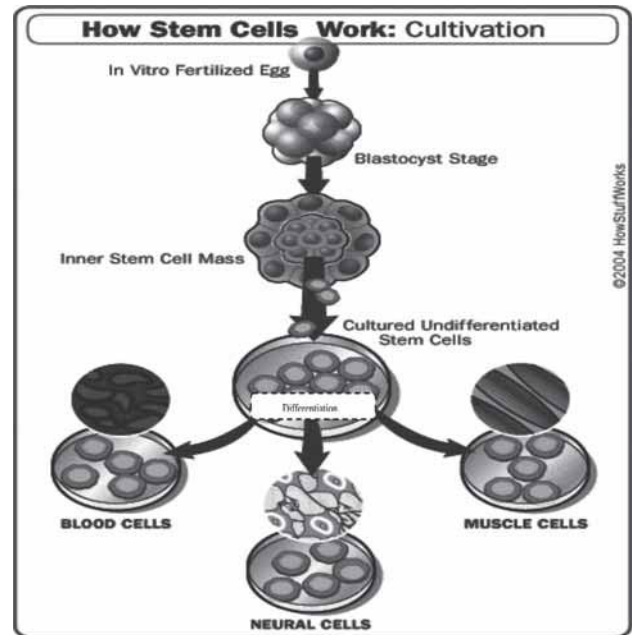
specialized cells, but also maintain the normal turnover of regenerative organs, such as blood, skin, or intestinal tissues.

There has been an explosive growth of information and speculation regarding the therapeutic potential of stem cells derived from adult and embryonic tissues.³²

A more conservative perspective focuses on the role of adult stem cells in wound repair. The present concept of stem cell differentiation is evolving, however, since progenitor cells from any tissues seem to be able to trans differentiate into other cell types when placed in an appropriate environment. DNA transfer must be carefully ruled out to validate these findings.

There are three categories of bone-marrow-derived cells that participate in the repair of connective tissue: (1) the angioblast or endothelial precursor cell (EPC); (2) the fibrocyte; (3) the marrow/mesenchymal stem cell (MSC). Epithelial layers, in general, harbour resident stem cells.

1.EPC-The Endothelial precursor cell is derived from a primitive haematopoietic cell in the bone marrow, prior to differentiation into the leucocyte lineage. It was first reported in 1997 as a cell type that could be



isolated from circulating blood, cultivated in vitro, transplanted in into syngenic host and localised to vascular structures.³³ Further work has decisively demonstrated that EPCs are recruited from the bloodstream at many sites of vasculogenesis, that is the de novo formation of new capillaries. Endothelial precursor cell are recruited to sites of repair or vessel growth by Vascular endothelial growth factor³⁴ and stromal-cell-derived factor (SDF).³⁵ These factors may also be involved in the mobilisation of the precursors from the marrow.³⁶ EPCs are not true stem cells, since they are apparently committed to the endothelial lineage while in circulation. For this reason, such cells can be purified from whole blood, based on their expression of the VEGF receptor 2 (flk-1) and the angiopoietin 1 receptor (tie-2). The haemangioblast and a more primitive progenitor, the multipotent adult progenitor cell (MAPC) have been suggested as the marrow-based precursors.³⁷

2.The Fibrocyte- First, described in 1994, is a leucocyte-like cell from bone marrow ,that infiltrates wounds during the inflammatory phase, produces collagen and has many characteristics of the antigen-presenting, dendritic cell.³⁸ This cell type can produce many cytokines, collagen and growth factors, and its presence has been associated with fibrotic conditions.

3.The Marrow/mesenchymal stem cell (MSC)- The MSC is another circulating, marrow-derived cell which is a pluripotential stem cell in that it can be isolated from marrow and grown for many generations in vitro, and MSC can be induced to differentiate into many types of mesodermal derivatives, including bone, cartilage, skeletal muscle and adipose tissue.³⁹ MSCs traffic to many different connective tissues and recent studies in a mouse model from this laboratory have shown that MSCs constitute a significant proportion of the collagen producing, fibroblastic population in a healing wound.⁴⁰

At present, it is not known whether these circulating sources of stem/precursor cells may be rate limiting for wound-healing processes. Patients undergoing immunosuppressive therapy are certainly at risk for healing problems due to infection, but marrow-derived mesenchymal cells may be more resistant. Ageing may affect the availability and regenerative capacity of stem cells. It is conceivable that we will be able to identify the factors that mobilize stem/precursor cells from the marrow and that stimulate their recruitment to sites of injury.⁴¹ There is not a great deal of

evidence that these marrow-derived cells take up permanent residence in tissues. They may be largely important during phases of acute repair where local proliferation cannot meet tissue needs.⁴⁰ It has recently come to light that many connective tissues do harbour pluripotential stem cell populations, including dermis,⁴² adipose⁴³ and skeletal muscle.⁴⁴ These may be alternative sources of stem cells for therapeutic applications.

Many epithelial tissues have much higher rates of cellular turnover and renewal, and resident stem cells are localised to specific areas. In the epidermis, stem cell populations have been identified in the bulge region of the hair follicle⁴⁵ and in the inter follicular zone.⁴⁶ The interfollicular cells represent a subset of the epidermal basal cells that undergo differentiation as they detach from the basal lamina and move towards the stratum corneum. While it has been difficult to identify specific surface characteristics that could aid in epidermal stem cell purification,⁴⁷ it is likely that these cells provide a significant fraction of the dividing keratinocytes in cultures that have been used to generate skin substitutes. There are several reports that indicate that these stem cells may be multipotent, and there are also reports that marrow-derived cells can be recruited through the bloodstream and participate in epidermal structure.⁴⁸ The clinical application of stem cells is well advanced for the treatment of corneal stem cell deficiency, chemical burns and several disease states.^{49,50}

Both unfractionated bone marrow as well as purified MSC from marrow and connective tissue sources have been evaluated in many forms of tissue repair: skin, bone, teeth, cartilage and tendon. There have been attempts to apply MSC to wounds: one study simply used whole marrow populations on three non-healing wounds with a favourable outcome⁵¹; another study reported improved healing on systemic injection of a dermal MSC population⁵²; there is also a report of MSC effects in deep burn wounds in rats. Favourable repair results have been obtained in bone and cartilage, and there is every reason to expect that living skin equivalents so engineered could enhance wound healing. Strategies that improve recruitment or growth of MSC may be effective.

Since the vascular supply is often rate limiting for repair, Endothelial Precursor Cells (EPC) also offer therapeutic potential.⁵² Agents that recruit Endothelial Precursor Cells, such as VEGF,³² also increase vascularity and other aspects of wound healing.³⁴ EPCs are readily purified from whole blood by apheresis techniques. Studies suggest that these cells and the factors that recruit them can reverse tissue ischemia. A study reports that purified human EPCs enhanced wound repair in the athymic nude mouse, increased vascularity and macrophage influx and occasionally became incorporated into patent, hCD-31 positive vessels.⁵³

SUMMARY

Gene transfer and applications of progenitor stem cells are two advanced technologies with great promise in wound healing and tissue repair applications. Safety issues have slowed the commercial development of gene transfer, but active trials are underway. This strategy is likely to overcome many of the drawbacks of recombinant proteins at potentially lower cost. Stem cell therapies with autologous grafting are likely to be accepted more easily by the medical and regulatory communities. Factors that regulate the mobilization, recruitment and differentiation of progenitor cells will also play an important role. Many of these findings will find their way into the development of more effective tissue engineering devices. The combination of gene transfer and applications of stem cells has even greater potential, since it lead to the design of medical devices that contain multipotential cells that are capable of delivering specific gene products.

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