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Review

Genetically-manipulated adult stem cells as therapeutic agents and gene delivery vehicle for wound repair and regeneration

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ABSTRACT

Wound therapy remains a clinical challenge and much effort has been focused on the development of novel therapeutic approaches for wound management. New knowledge about the way in which signals control wound cellular and molecular behavior has promoted the topical application of multipotent stem cells and bioactive molecules to injured tissue, for skin regeneration with less scar formation. However, limited clinical success indicates that the effective delivery of polypeptides and therapeutic cells, with controlled releasing profile, is a major challenge which is yet to be overcome. Recently, a technique in which the genetically-manipulated stem cells were used both as the therapeutic agents and the vehicle for gene delivery for wound treatment – a method which serves to provide regenerative cells and bioactive genes within an optimal environment of regulatory molecular expression for wound sites – has emerged as a promising strategy for wound regenerative therapy.
In this article, the roles of adult stem cells – as the therapeutics and the vehicles in these advanced biomimetic drug delivery systems for wound regeneration medicine – are scrutinized to indicate their mechanisms, characteristics, broad applicability and future lines of investigation.

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1. Introduction

Skin plays an extremely important role in providing an immune network and physical barrier against mechanical, chemical, and microbial factors from the external environment, as well as acting as a unique defense system against UV radiation (UV-R) (ii) through its pigments, secreted by melanocytes located in the bottom layer of epidermis. The effective healing of deep dermal wounds – including those which are chronic in nature or slow to heal – and the avoidance of scarring complications are challenging objectives which illustrate a general lack of efficient treatments for large wounds [1]. Emerging knowledge of the cellular activities involved in the wound healing process has advocated the topical delivery of large number of therapeutic cells, e.g. keratinocytes and fibroblasts, after propagation *ex vivo* for (i) the immediate coverage of the wound site, thereby stimulating the host to produce a variety of cytokines, (ii) the provision of the basement membrane, (iii) the prevention of dehydration and (iv) the activation of healing responses [2]. However, major problems have occurred, over the past years, with the topical delivery of cells or/and growth factors using traditional techniques, such as: (i) the lack of long-term integration of the cellular sheets, (ii) the incomplete healing and frequent generation of scar tissue, (iii) the inadequate appendage contribution and (iv) overt rejection.

Meanwhile, reduction in tissue growth factors and cytokines has been shown to significantly delay wound healing and to initiate scar formation [3], which stimulated the widespread application of growth factors in wound therapy. However, the clinical effectiveness of traditional topical administration of growth factor peptides remained discouraging, suffering from the inherent loss of drug activity, due to the combined effects of physical inhibition and biological degradation of the bio-molecules.

Recently, gene transfer technology – in which genetically-modified cells synthesize and deliver the encoding growth factors to the wound site in a time-regulated and locally-restricted manner – has been experimentally demonstrated to be a valuable means of overcoming the limitations associated with traditional, topical delivery of recombinant proteins [4].

More interestingly, in recent years, the use of genetic recombinant stem cells and biomimetic nanostructured scaffolds for the development of novel biomimetic drug delivery systems, has received widespread attention as a promising strategy for wound treatment, in which multipotent stem cells, encoded with plasmid DNA of polypeptides, are used both as the cellular therapeutic medium and the vehicle for the delivery of functional genes to the wound site. In this article, the roles of bioactive molecules and tissue-specific adult stem cells involved in wound healing are reviewed, followed by a discussion of their application in the construction of biomimetic drug delivery systems for skin repair and regeneration.

2. Functions of bioactive molecules in wound healing

2.1. Bioactive molecules in wound healing

Normal wound repair of tissues and organs involves four overlapping yet distinct stages. These are: (i) the initiation of hemostasis and inflammatory response; (ii) the formation of granulation tissue, epithelialization and angiogenesis, as well as the synthesis of extracellular matrix proteins; (iii) the maturation and remodeling of connective tissue and (iv) the recovery of the wound strength, accompanied with fibrosis (see Fig. 1 as an overview of the wound healing process). It can be seen that wounds include a complex signaling network, which involves numerous molecules. The cellular and molecular mechanisms which critically influence the wound repair quality, and how their related responses can be manipulated by therapeutic intervention, represent key questions in wound genetic- or cellular therapy. Currently, many experimental studies have helped to clarify the influences, on wound repair and healing quality, of growth factors, cytokines, chemokines, transcriptional factors and newly-identified bioactive molecules [5–7]. (Fig. 1 showed a list of the participation and up-regulation in most conditions of the bioactive molecular effectors in the wound healing stages of inflammation and hemostasis, re-epithilialization, angiogenesis and remodeling.) However, a critical issue in the application of these functional molecules in wound healing has been the development of a strategy to optimize the delivery of these polypeptides, in order to maximize their therapeutic efficacy. In recent years the molecular genetic approach – using genetically-manipulated cells as the vehicle for delivery of the desired growth factor – has been experimentally demonstrated to be a useful strategy. Also, in contrast to employing many differentiated cell phenotypes, stem cells are potentially permanent residents of the wound site and will help to sustain the expression of the transfected genes and bioactive proteins. This knowledge may create new opportunities for the clinical application of versatile molecular regulators in tissue healing.

3. Adult stem cells involved in wound repair and regeneration

Besides emerging knowledge about the participation of various molecular effectors, the identification of the roles that stem cells play in wound regeneration is probably the most active field in wound healing mechanistic investigation. Currently, stem cells derived from epidermis, dermis, hair follicle, adipose tissue and bone marrow have been demonstrated to contribute significantly to the maintenance of skin renewal, homeostasis and regeneration. Their functions in wound healing include: (i) acting as multiple players in re-epithelialization; (ii) being progenitor cells with differentiation potential from mesenchymal to epithelial phenotypes; (iii) being endothelial cell precursors for revascularization; (iv) expressing the fibroblast-specific collagen genes that contribute to wound remodeling; (v) promoting repigmentation; and (vi) causing hair follicle neogenesis [8,9]. The characteristics and roles of the cutaneous stem cells that are closely involved in wound healing are reviewed as follows.

3.1. Epidermis and hair follicle-derived stem cells

The maintenance of homeostasis in the epidermis is possible via the self-renewing ability of the epidermal stem cell (ESC) population, which gives rise to differentiated keratinocytes. It is believed that ESCs play an important role in cellular regeneration, wound healing, and the pathogenesis of skin cancers. ESCs reside in the basal layer of the epidermis and are known to be responsible for regenerating the epidermal tissue during an individual's lifetime because of their great proliferative capacity. Under physiological circumstances, ESCs serve as a cell pool for the cyclic regeneration of the anagen hair bulb and they also regenerate the sebaceous gland and the epidermis after injury [10,11]. The bulge region of the hair follicle is firmly believed to be a niche of multipotent stem cells. Subsets of these follicle-derived multipotent stem cells mainly include the hair-follicle-derived epithelial stem cells, mesenchymal stem cells, melanocyte and nestin-positive stem cells. These stem cells can be activated and they then migrate out of the hair follicles to the site of a wound, to replenish lost cells and repair the damaged epithelium, the follicle and the sebaceous gland [12]. Hair-follicle stem cells (HFSCs) are neither necessary for epidermal survival, nor does their absence prevent re-epithelialization, but they seem to enhance the early stages of wound closure [13]. Additionally, there is increasing evidence that HFSCs are a source of epidermal and dermal cell populations. However, it is surprising to find that the majority of HFSCs do not persist in the regenerated epidermis and this may suggest that HFSCs and ESCs are intrinsically different, in that the ESCs seem better suited to establishing long-term epidermal units [14]. Because of the marked ability