Research Journal of Medical Sciences 10 (4): 296-301, 2016
ISSN: 1815-9346


# Understanding the Signaling Pathway as a New Strategy for Burn Management and Treatment Based on Stem Cell Therapy

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**Abstract:** Burn injury has been reported to be an important cause of morbidity and mortality in many countries which leads to a loss of integrity of the skin which protects us from water loss, temperature change, radiation, trauma and infection. The main sources of stem cells that might be used for repair and regeneration of injured skin tissue are Embryonic Stem Cells (ESCs) and adult stem cells. ESCs have a great capacity for self-renewal and pluripotency but their clinical applications are limited because of the political and ethical considerations. Also, ESCs themselves are less suitable for tissue grafting; they do provide the potential to augment physiological healing processes via paracrine mechanisms. Stem cells allows for the possibility of restoring lost or damaged tissue, while their ability to modulation of immune system the wound bed from afar suggests that their clinical applications need not be restricted to direct tissue formation. The clinical utility of stem cells has been demonstrated across dozens of clinical trials in chronic wound therapy; thus, it promotes normal interactions between cell assemblies during the regeneration of burn wounds which prevents the formation of cicatrix or the deformation of tissues. Furthermore, Induced Pluripotent Stem Cells (iPSCs) derived fibroblasts may be an increased production of extracellular matrix proteins that could also increase the rate of wound healing as well as provide opportunities for eventually generating these structures without the risk of immune rejection.

**Key words:** Stem cell, regenerative medicine, burn healing, cell interaction, pluripotent stem cells

## INTRODUCTION

Burn injury is a devastating trauma the most common and devastating forms of trauma that having a great impact on the patients physically, physiologically and psychologically (Van Loey and van Son, 2003). Patients with serious thermal injury require immediate specialized care in order to minimize morbidity and mortality thus survival rates are increasing, burn injury remains a great challenge in the field of cutaneous wound healing (Rowan *et al*., 2015). Cultured Epithelial Autograft (CEA) are the prototypical cell-based therapy in which keratinocytes are grown in a sheet and applied to a wound which most burn patients lack enough skin to treated with CEA are still neither efficient nor effective solutions (Atiyeh and Costagliola, 2007). Transplanted skin from donors is currently not an option due to rejection; however, augmenting immunotolerance via Stem Cell (SC) therapy may overcome this problem. Regenerative medicine using SCs is an efficient, low-morbidity and high-quality therapy for skin coverage in burns, mainly due to the regeneration of skin appendages and the minimal risk of hypertrophic scarring (Huang and Burd, 2012). Burn injury represents a cellular stress in the skin. Normal adult skin repair is slow, with high risk of infection and hypertrophic scarring. Epidermal keratinocytes form

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a scar without cutaneous appendages, such as hair follicles, sweat or sebaceous glands, thus capable of contributing to epithelial regeneration across the wounded surface. In addition, the hair follicles of human skin contain a reserve of stem cells, located in the bulge region of the follicle which are capable of self-renewal (Roh and Lyle, 2006). In order to, SCs enhance the process of burn wound healing via blood stream with identical phenotypes to mesenchymal bone marrow SCs after acute large skin burns (Rowan *et al*., 2015; Mansilla *et al*., 2006). Hence, it was concluded that these SCs may have a role in promoting wound healing in burns, probably increased levels of bone marrow derived endothelial progenitor cells in burn patients can be promote burn wound repair by paracrine signaling (Borena *et al*., 2015; Abedi *et al*., 2012). These levels were proportional to the extent of the burn. The study also showed increased levels of angiogenic cytokines which may be involved in the signaling pathway for promoting the release of bone marrow derived SCs (Duscher *et al*., 2015). Focusing on the role of cytokines in burn wound healing, Payne *et al*. (2010) studied the role of amnion derived cellular cytokine solution. Some studies show using the amnion derived multipotent progenitor cells to harvest cytokines can be reliable strategy for burn wound healing (Duscher *et al*., 2015; Ojeh *et al*., 2015). Amnion derived cellular cytokine solution showed statistically significant improvement in the epithelialization of the burn wounds and the appearance of hair growth compared to controls (Payne *et al*., 2010). There was an increased level of endothelial progenitor cells in the bloodstream as well as its capillary permeability after skin burns and escharectomy, posing a possible role of escharectomy in burn wound healing (Foresta *et al*., 2011). Many people argue that embryonic SC harvesting would be done by killing embryos which would be unethical. Others would argue that even if embryos are used for SC research, it is not wrong (Lo and Parham, 2009). The extent of inflammation and hypermetabolism is related to the extent and depth of burn, as deeper burns show higher levels of circulating cytokines and a greater hypermetabolic response which varied based on the understand immune responses in different burn depths may produce knowledge about the pathophysiology of major burns (Williams *et al*., 2009). The mechanisms underlying apoptosis and necrosis in the ischemic zone remain poorly understood but appear to involve immediate autophagy within the first 24 h following injury and delayed-onset apoptosis may be occurrence involved in burn wound progression as well as one day after injury (Xiao *et al*., 2014; Farzianpour *et al*., 2014). Oxidative stress may play a role in the development of necrosis, as preclinical studies have demonstrated promising reductions in necrosis with systemic antioxidant administration. At the outermost regions of the burn wound is the zone of hyperemia that receives increased blood flow via inflammatory vasodilation and will likely recover, barring infection or other injury (Atiyeh and Costagliola, 2007; Xiao *et al*., 2014). To repair and re-epithelialize a wound, SCs from the hair follicle bulge give rise to daughter skin cells which migrate to the epidermis (basal layer and sebaceous gland). In adult skin, superficial burns that leave hair follicles intact are healed rapidly with the regeneration of epidermal appendages (Blanpain and Fuchs, 2006). SCs therapy aims to accelerate reepithelialization after burn injury via expression and activity of collagenases is tightly controlled by cytokines, like TGF-βI. It's also to reconstruct functional skin with sweat glands; hair follicles and dermal capillaries through inhibit the production of matrix proteinases and stimulate the production of proteinase inhibitors (Jeschke *et al*., 2013). Approaches to SC therapy include local recruitment of endogenous SCs transplantation, either of which can be combined with gene therapy or tissue engineering. Tissue engineering is perceived as a better approach because the repair process may proceed with the patient's own tissue by the time the regeneration is complete as well as can be an experimental method that combines cellular biology, engineering and medicine to develop three-dimensional tissues and restore or establish normal function (Metcalfe and Ferguson, 2007).

## MATERIALS AND METHODS

**Stem cell types and classifications:** A stem cell is an unspecialized cell that can divide without limit as needed and can, under specific conditions, differentiate into specialized cells. Stem cells are divided into several categories according to their potential to differentiate. Furthermore SCs are defined by two main characteristics: their capacity of prolonged self-renewal and multilineage differentiation (asymmetric replication) (Huang and Burd, 2012; Duscher *et al*., 2015) (Fig. 1). These characteristics are more pronounced in younger sources. By asymmetric replication, after every cell division, one cell retains its self-renewing capacity, while the other (Transit-Amplifying or TA cell) enters a differentiation pathway and joins a mature non-dividing population (Duscher *et al*., 2015; Ojeh *et al*., 2015). When an unspecialized SC differentiates, it assumes characteristics of a specific tissue. SCs are pluri, multi or unipotent. The zygote is the only mammalian cell capable of producing all cells and tissues of an organism and thus is considered totipotent (Cerqueira *et al*., 2016).

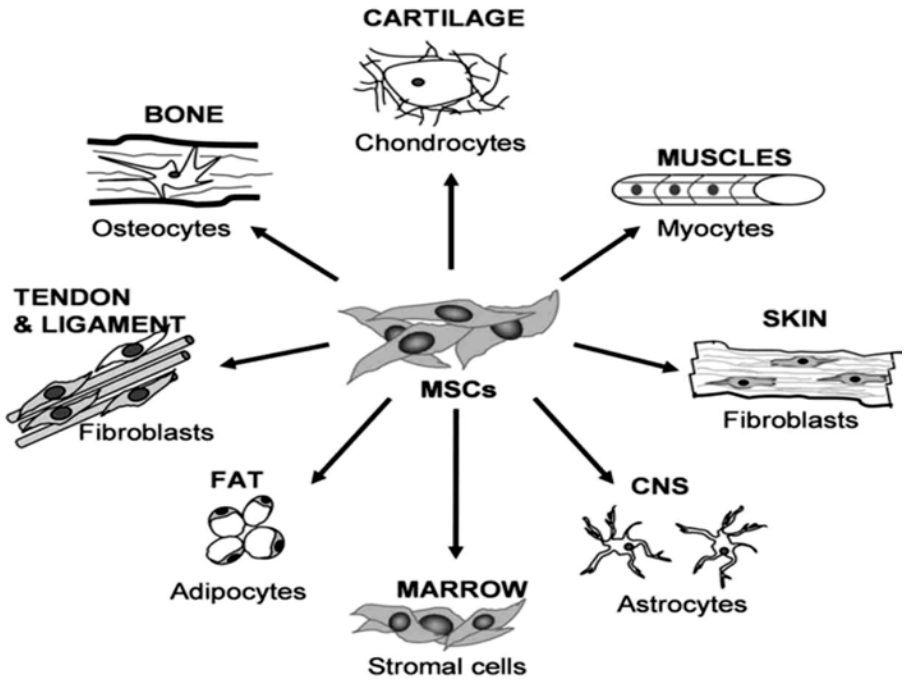


Fig. 1: Multilineage differentiation potential of Mesenchymal Stem Cells (MSCs). Under appropriate conditions, MSCs are able to differentiate into cell types of different lineages, including cartilage (chondrocytes), bone (osteocytes), marrow (stromal cells), muscle (myocytes), Fat (adipocytes), tendon (fibroblasts) and skin (fibroblasts)

Table 1: Comparison of embryonic and adult stem cells in terms of biological characteristics and differentiation potential

| ` | Type | |
|---|---|---|
| Properties | Embryonic stem cells | Adult stem cells |
| Differentiation ability | Pluripotent stem cells can become all cell types of the body | Multipotent stem cells limited to differentiating into different cell types of their tissue |
| Culture | Grown easily in culture | Isolation and expansion challenging |
| Rejection after transplantation | Do not cause rejection | Cause rejection |
| Tumour formation after injection | Tumour genesis | Is not tumour genesis |
| Colony forming | Form embryoid bodys | MSC form colony forming units<br>NSC form neurispheres |

Pluripotent cells produce cells and tissues belonging to all three germ layers include ectoderm, mesoderm and endoderm. Multipotent cells produce more than one cell lineage, within a closely related family of cells. Unipotent cells only differentiate into a single cell phenotype. Plasticity describes the phenomenon whereby SCs from one tissue produce cell types of a completely different tissue (Huang and Burd, 2012; Ojeh *et al*., 2015). When classified by their origin and location in the human body, there are two types of SCs: Embryonic (ESC) and adult (non-embryonic) SCs that can be isolated from various sources including embryos, fetal tissues, umbilical cord blood and adult organs (Lodi *et al*., 2011) (Table 1).

**Mesenchymal SCs:** Mesenchymal SCs (MSCs), also referred to as mesenchymal stromal cells, can be isolated from bone marrow and many other sources, such as adipose tissue, umbilical cord and cord blood (Mansilla *et al*., 2006). MSCs are the most promising and substantially evaluated SC type for their plasticity in tissue repair and regeneration (Mansilla *et al*., 2006, Duscher *et al*., 2015). MSCs have the capability to differentiate into cells with the mesodermal, ectodermal and endodermal characteristics. They contribute to wound repair and regeneration through direct differentiation or transdifferentiate into tissue-specific cells to reconstitute the tissue; they also release paracrine factors to stimulate the survival and functional recovery of the resident cells (Duscher *et al*., 2015, Ojeh *et al*., 2015). MSCs are found to have immune-regulatory potency in addition to their low immunogenicity feature (Huang and Burd, 2012). Their regulatory capacity enables

the modulation of the local microenvironment or niche and the host immune response. As a result, enhanced angiogenesis and suppressed immune response are often observed after MSCs administration. These hold fundamental implications for potential therapeutic applications in burns and wounds (Teng *et al*., 2014). The clinical application of Bone Marrow MSCs (BM-MSCs) has been demonstrated in both acute and chronic wounds. In the treatment of diabetic foot ulcers, the combined therapy with topical application and edge injection of the bone marrow aspirates together with further application of culture-expanded BM-MSCs obtained successful closing and healing of the previously non-healing ulcers (Duscher *et al*., 2015, Maxson *et al*., 2012). BM-MSC therapy has also been demonstrated with success in both preclinical and clinical settings for treating a particular burn type, the radiation burns. With local injection of culture-expanded autologous BM-MSCs, the clinical evolution of radiation-induced complications were significantly improved (Domergue *et al*., 2016). Bone marrow contains hematopoietic Scs and non-hematopoietic SCs; it releases a variety of angiogenic, antiapoptotic and mitogenic factors which are all important mediators in wound healing (Chen *et al*., 2013). In recent years a significant progress with the plasticity of MSCs in skin regeneration is the demonstration of its potential to regenerate the sweat gland. Human BM-MSCs co-cultured with heat-shocked Sweat Gland Cells (SGCs) exhibited a phenotype conversion from MSC to SGC; the Extracellular Signal-Regulated Kinase (ERK) pathway was found to play an important role in the differentiation (Zhang *et al*., 2007). In full-thickness burns, sweat gland cannot be regenerated by healing. The success in regenerating functional sweat gland from MSC transplantation would provide a significant benefit for patients surviving extensive deep burns (Sheng *et al*., 2009; Abedi *et al*., 2012). Adipose-Derived SCs (ADSCs) similarities with MSCs isolated from bone marrow, thus via releasing the cytokine and growth factor have several beneficial effects in inflammatory and autoimmune diseases. Furthermore, ADSCs due to the relative ease of access, high cell yield and putative its anti-inflammatory effects make them attractive targets for skin engineering which ability to differentiate into several cell lineages that's to be applied in skin regeneration strategies (Kim *et al*., 2009; Abed *et al*, 2011).

## RESULTS AND DISCUSSION

**Embryonic SCs (ESCs) and Induced Pluripotent SCs (IPSCs):** Embryonic stem cells are derived from the inner cell mass of blastocyst stage embryos are pluripotent SCs and give rise to all cells of the three embryonic germ layers: endoderm, mesoderm and ectoderm (Duscher *et al*., 2015; Ojeh *et al*., 2015). Human Embryonic SCs (HESCs) are derived from excess developing pre-implantation embryos (5 day-old embryos, 4-8 day-old morula or inner cell mass of blastocysts) that have usually been fertilized in vitro at a fertilization clinic (Ojeh *et al*., 2015; Chen *et al*., 2009). Derivation of human embryonic cell lines is controversial because it requires destruction of an embryo, may develop teratocarcinoma (tumors composed of tissues from all three germ layers), are immunogenic and show genetic instability *in vitro* (Branski *et al*., 2009; Teng *et al*., 2014). ESCs are capable of unlimited expansion in vitro and are considered an immortal epiblast derivative with a checkpoint in differentiation that enables their expansion as undifferentiated colonies which can be instructed to maintain this phenotype indefinitely (Metcalfe and Ferguson, 2007; Butler *et al*., 2010).

Induced Pluripotent SCs (iPSCs) are artificially derived from non-pluripotent cells, typically adult somatic cells (mostly fibroblasts of murine or human origin) and most frequently by epigenetic reprogramming and also by nuclear transfer or cell division (Sun *et al*., 2014). Expression of transcription factors characteristic for undifferentiated embryonic SCs is induced; for example, OCT4 (also known as POU5F1, being the most important one), SOX2, c-MYC, KLF4, Lin28 and/or NANOG (Patel and Yang, 2010). Transcription factors or cell markers are the key mediators of cellular identity. Direct reprogramming (also referred to as transdifferentiation) describes ectopic expression of defined transcription factors, a very slow and inefficient process that may limit the quality of resulting iPSCs (Ko *et al*., 2011). For example, fibroblasts can be reprogrammed into neurons, cardiomyocytes and blood-cell progenitors. Small molecules may improve the reprogramming efficiency but increase its tumorigenicity. Elements that influence reprogramming include the respective donor cell type, the transcription or reprogramming factors utilized, the mode of delivery (e.g., virus, RNA, etc.) and the culture conditions, all of which depend on the purpose of the process (Kelaini *et al*., 2014).

## CONCLUSION

The various clinical challenges in treating acute thermal injuries include balancing the many factors that affect wound healing to reduce the length of stay (and associated cost of treatment), the risk of infection, the time to wound closure and the overall time to functional

recovery. The treatment of burn wounds has evolved over several decades through clinical and preclinical research. Significant advancements have been made in patient care, including tracking wound healing, developing novel graft and coverage options, controlling inflammation, optimizing dietary needs and testing unique pharmacological interventions. SC-based therapy has offered a novel and powerful strategy in almost every medical specialty including burns and wound management. SCs have proven to have tremendous potential in enhancing wound healing and facilitating skin regeneration. Additionally, the signaling pathways followed by SCs involved in the burn wound healing along with their factors and signals constitute a very dynamic and promising research field. The choice of suitable SC sources in sufficient quantity, adequate culture conditions to preserve SC property, appropriate matrices or scaffolds to improve cell delivery efficiency will all have a great impact on the clinical outcomes of SC application. In terms of the anticipated clinical practice, SC therapy has to be improved to the point that hospital can put safe, efficient and reliable protocols into practice.

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