SHORT COMMUNICATION

Stem cells conditioned medium: a new approach to skin wound healing management

Pukana Jayaraman¹, Prakash Nathan², Punitha Vasanthan¹, Sabri Musa¹* and Vijayendran Govindasamy²,³*

¹ Department of Children’s Dentistry and Orthodontics, Faculty of Dentistry, University of Malaya 50603, Kuala Lumpur, Malaysia
² Hygieia Innovation Sdn Bhd (852106-M), Lot 1G-2G, Lanai Complex No. 2, Persiaran Seri Perdana, Precint 10, Federal Territory of Putrajaya Malaysia
³ KOZA Sdn Bhd (947767-T), 13.3A, Menara 1 Mont Kiara, Jalan Kiara, 50480 Mont Kiara, Kuala Lumpur, Malaysia

Abstract

Stem cell biology has gained remarkable interest in recent years, driven by the hope of finding cures for numerous diseases including skin wound healing through transplantation medicine. Initially upon transplantation, these cells home to and differentiate within the injured tissue into specialised cells. Contrariwise, it now appears that only a small percentage of transplanted cells integrate and survive in host tissues. Thus, the foremost mechanism by which stem cells participate in tissue repair seems to be related to their trophic factors. Indeed, stem cells provide the microenvironment with a wide range of growth factors, cytokines and chemokines, which can broadly defined as the stem cells secretome. In in vitro condition, these molecules can be traced from the conditioned medium or spent media harvested from cultured cells. Conditioned medium now serves as a new treatment modality in regenerative medicine and has shown a successful outcome in some diseases. With the emergence of this approach, we described the possibility of using stem cells conditioned medium as a novel and promising alternative to skin wound healing treatment. Numerous pre-clinical data have shown the possibility and efficacy of this treatment. Despite this, significant challenges need to be addressed before translating this technology to the bedside.

Keywords: cytokines; growth factors; host tissues; paracrine activities; regenerative medicine; tissue repair

Introduction

Statistics reported by the World Health Organization (WHO) estimated that each year over 300,000 people die of skin injury, with the highest death documented in South-East Asian countries (Mock, 2007). In general, skin wound healing takes around 2 weeks depending on the wound severity (acute or chronic; Szpaderska et al., 2003; Figure 1). The slow recovery of natural wound healing has resulted in the entry of exogenous wound healing treatments. Since then, many treatments have proved to quicken the healing (Figure 2). Nevertheless, cost build-up and inconsistency in healing are the major pitfalls of these treatments. This resulted in the discovery of more advanced treatments, such as tissue engineering (Chen et al., 2009), gene therapy (Song et al., 2012), platelet-rich plasma (Park et al., 2011), growth factors (GF) (Penn et al., 2012) and stem cells (SC) therapy (Lee et al., 2012). Among these, SC has become the centre of attraction in wound healing by promoting microvascular remodelling (Dulmovits and Herman, 2012) and enhancement of neovascularisation (Choi et al., 2013). Many approaches can be envisioned for using SC in the support of wound healing. Obviously the first approach that comes to the mind is the injection of SC directly into the wound; reports have shown that SC plays a major role in strengthening wound healing by secreting a multitude of trophic and survival signals including GF, chemokines and cytokines (Chen and Tredget, 2008). They serve as a tool among cells to communicate and these molecules can be traced in the conditioned medium (CM) or spent medium harvested from cultured cells. Conditioned medium has been used in pre-clinical studies as a substitute for numerous cellular based therapies including wound healing (Walter et al., 2010). This has encouraged the use of CM in wound healing by modulating wound repair without SC being present in the wound. Nonetheless, details of this method remain uncertain and must be proved before taken as fact.

Corresponding author: e-mail: sabrim@um.edu.my (S. Musa); vijay@hygieiagroup.com (V. Govindasamy)
Figure 1 Wound healing stages.

Inflammatory Phase
After tissue injury, a coagulation cascade is initiated to stop bleeding.
In the presence of infection, the neutrophils increased.

Late-Inflammatory Phase
The macrophages stimulate angiogenesis and re-epithelialization.
Fibroblast activated to deposit excessive amount of collagen for wound repair.

Proliferation Stage
Granulation tissue begins to form and is a loose network of collagen, fibronectin and hyaluronic acid.

Maturation Phase
Further collagen deposition and cross linking of extracellular matrix will occur therefore the scar tissue gains tensile strength.
Figure 2 Treatment modalities for wound healing.
Cell free therapy: an alternative in wound healing management?

One of the major limitations of SC based treatment is the low survivability of cells after being transplanted in the host (Modo et al., 2002). In addition, there are reports suggesting similar characteristics exist between mesenchymal stem cell (MSC) and cancer SC (Kucia et al., 2005). There is even evidence suggesting that SC within normal tissues are of cancerous origin (Sell, 2010). Therefore, to ensure the safety of SC based therapies, developing an alternative approach to direct transplantation of stem cells is necessary. The use of SC-CM instead of direct implantation of SC perhaps offers a better solution to overcome the limitation of cell based therapy.

Lee et al. (2011) reported that CM of human embryonic stem cell (hESC)-derived endothelial precursor cells (EPC) containing high level of GF and cytokines such as epidermal growth factor (EGF), basic fibroblast growth factor (bFGF), fractalkine, granulocyte-macrophage colony-stimulating factor (GM-CSF) and interleukin (IL)-6 were successfully used in the treatment of excisional wound healing in rats. Chen and Tredget (2008) suggested a significantly increased wound closure using bone marrow. Oesenchymal stem cell (BM-MSC)-CM compared to fibroblast-CM which promises novel therapies for wound repair. They reported that BM-MSC-CM secreted higher paracrine factors such as vascular endothelial growth factor (VEGF)-α, insulin like growth factor (IGF), EGF, keratinocyte growth factor (KGF), angioprotein-1 (Ang-1), stromal derived factor-1 and erythropoietin (EPO) compared to fibroblast-CM, indicating that the origin of cells are significantly contributing to the production of paracrine factors. Adipose derived stem cell (ADSC)-CM also has regenerative effects on skin wounds. It stimulates both collagen synthesis and migration of dermal fibroblasts hence promoting wound healing and improving wrinkling in animal models (Kim et al., 2009). ADSC-CMs upregulate the transcription of type I procollagen-alpha-1 chain gene of fibroblasts and involve Rho-associated kinase (RhoA-ROCK) signalling, which leads to the proliferation of keratinocytes and dermal fibroblasts. Dental pulp stem cell (DPSC)-CM has the ability to enhance wound healing by increasing collagen synthesis, and activating proliferation and migration activity of human dermal fibroblast (HDF) (Ueda and Nishino, 2010). Inoue et al. (2013) reported that DPSC-CM enhances vasculogenesis, migration and differentiation of endogenous neuronal progenitor cells in ischemic brain injury in a rat model.

How does SC-CM works in wound healing?

Skin injury causes blood vessel damage and leakage of blood constituents into the wound site. Hemostasis begins immediately after wounding, with vascular constriction and formation of fibrin clot (Szpaderska et al., 2003). As depicted in Figure 1 in the natural wound healing process, the migration of inflammatory cells into the wound by chemotaxis starts with the infiltration of neutrophils, macrophages and lymphocytes (Gosain and DiPietro, 2004). These cells are a major source of GF through phosphoinositide 3-kinase (PI3K) PI3K/Akt, and Janus kinase and Signal Transducer and Activator of Transcription (Jak-STAT) pathways. For example, neutrophils initiate VEGF and transforming growth factor-β (TGF-β), whereas lymphocytes initiate tumour necrosis factor (TNF), granulocyte-colony stimulating factor (G-CSF), granulocyte macrophage-colony stimulating factor (GM-CSF) and IL-1 and macrophage secretes bFGF, EGF, platelet-derived growth factor (PDGF), GM-CSF, TGF-α, TGF-β, IL-1 and TNF. Depending on the individuals, the expression of these groups of cytokines and GF determine the duration of wound healing process. At this juncture, the introduction of SC-CM to the site of injury may accelerate the recovery process. This is because, apart from host tissues, SC-CM has a wide range of cytokines and GF related directly to the wound healing process (Table 1). The next phase of recovery involves angiogenesis, whereby molecules such as VEGF, bFGF, EGF and TGF-β promote new blood vessel, sustain the newly formed granulation tissues and help in the survival of endogenous keratinocytes. In the late phase of the wound healing process, GFs such as EGF, GM-CSF and hepatocyte GF (HGF) prompt keratinocytes to migrate from the basal population around the wound edge to cover the lesion and differentiate into squamous keratinizing epidermal cells (Metcalfe and Ferguson, 2007; Figure 3.

Challenges to SC-CM therapy

Numerous questions remain to be answered before SC-CM can be used as an efficient therapeutic tool, the key ones being addressed below.

Secretome factors

The level of paracrine factors secreted by different SC resources plays an important role on their influences on cell recruitment and wound repair (Friedenstein et al., 1966). Hence, the question is how to increase the paracrine factors in SC-CM enough for them to be used for the treatment. Hypoxia treatment is perhaps one of the ways. Hypoxic stress is a condition that reduces oxygen, which will improve cellular functions depending on the cell type, position and microenvironment. When ADSC are cultured under hypoxic conditions in vitro, the proliferative and self-renewal capacities of the cells are significantly improved, enhancing the secretion of certain GFs (Efimenko et al., 2010). Kinnaird et al. (2004) and Lee et al. (2009) reported a wide variety of...
cytokine genes expressed in MSC-CM collected in hypoxic condition; it has promoted in vitro proliferation and migration of endothelial cells as well as collagen synthesis. Another aspect is the timing of collection of the CM from the cells. Walter et al. (2010) showed that CM collected and filtered after 72 h incubation from a population of MSC at passage II used to replace CM in scratch wound assay medium successfully improved the healing ability in this assay.

**Choice of cells**
MSC derived from various tissue sources are different from each other, indicating their propensity towards a specific lineage (Pal et al., 2009; Nekanti et al., 2010). Similarly, we suggest that there will be variation in terms of cytokine and GF among various cell sources whereby the right source needs to be identified to provide maximum efficacy in wound healing treatments.

**Safety issues**
Stem cells culture is usually expanded in basic media with fetal bovine serum (FBS) or other serum supplements such as human platelet lysate (HPL) (Lohmann et al., 2012). The collection of SC-CM with serum supplemented condition method may not be adequate as it can introduce animal derived cytokines and GFs to the medium. For better therapeutic usage of SC-CM, the use of completely defined serum-free conditions is desirable, but

### Table 1 List of cytokines secreted by SC-CM.

| Paracrine factors | Function | Phase of wound |
|-------------------|----------|----------------|
| Transforming growth factor beta (TGF-β) | Stimulates migration of macrophages, dermal fibroblasts. Increases angiogenesis and granulation tissue for re-epithelialisation process | Inflammatory, proliferation |
| Transforming growth factor-alpha (TGF-α) | Stimulates epithelial cells and granulation tissue for re-epithelialization process | Inflammatory, proliferation |
| Basic fibroblast growth factor (bFGF) | Increases fibroblast proliferation, angiogenesis and matrix deposition | Proliferation, maturation |
| Interleukin-6 (IL-6) | Influencing inflammatory cells influx and promotes reepithelialisation | Inflammatory, proliferation |
| Interleukin-8 (IL-8) | Promotes skin re-epithelialisation by increasing keratinocyte migration and proliferation | Proliferation |
| Interleukin-1 (IL-1) | Increases pro-inflammatory cell and fibroblast proliferation | Inflammatory, proliferation |
| Epidermal growth factor (EGF) | Enhance migration of keratinocyte and fibroblast. Increased granulation tissue | Proliferation |
| Vascular endothelial growth factor (VEGF) | Endothelial survival and migration and proliferation. Regulates angiogenesis and granulation tissue formation | Inflammatory, proliferation |
| Platelet-derived growth factor (PDGF) | Increase macrophage activation, fibroblast proliferation, angiogenesis and collagen metabolism | Inflammatory, proliferation, maturation |
| Keratinocyte growth factor (KGF) | Stimulation of keratinocytes’ proliferation and migration | Proliferation |
| Granulocyte-colony stimulating factor (G-CSF) | Initiate inflammatory cells and increases keratinocytes | Inflammatory, proliferation |
| Granulocyte macrophage-colony stimulating factor (GM-CSF) | Proliferation of epidermal cell | Proliferation |
| Tumour necrosis factor (TNF) | Increases fibroblast | Proliferation |
| Insulin like-growth factor (IGF-1) | Fibroblast and collagen synthesis | Proliferation, maturation |
| Hepatocyte growth factor (HGF) | Promotes reepithelialisation, vasculogenesis and granulation tissue formation | Proliferation |
| Macrophage chemotactic protein-1α (MCP-1) and RANTES | Promote dermal wound healing as a chemoattractant to cells of the immune system particularly macrophages | Proliferation |
| Collagen type 1 and fibrinectin | Stimulates fibroblast and keratinocyte cell adhesion and migration | Maturation |
| SPARC | Cell-matrix interaction | Maturation |
| Insulin-like growth factor binding protein 7 (IGFBP-7) | Regulate proliferation and migration of keratinocytes | Inflammatory, proliferation |
| Connective tissue growth factor (CTGF) | Chemo attractant for fibroblast | Proliferation |
may escalate production cost. There is also the possibility of introduction of dead cells and extracellular matrix well as cell debris in SC-CM.

**Delivery method**

Multiple combinations of administration routes are perhaps the best in the case of SC-CM. In a wound healing model, Chen and Tredget (2008) gave each excision wound 80 μL MSC-CM by subcutaneous injection and 20 μL by topical application on the bed, and showed remarkable recovery (Friedenstein et al., 1966).

**Conclusion**

It is clear that SC-CM technology is a rapidly advancing field that promises to have a substantial impact on the treatment of skin wound healing. Therefore, gaining a more complete understanding of growth factors and cytokines in the SC-CM is crucial, together with finding better solutions to some of the key questions we have raised. In addition, knowledge of SC-CM could persuade academic, pharmaceutical and regulatory scientists to agree on a common path forward that will maximize the possibility of clinical realization of SC-CM therapies.

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**Conflict of interest**

None.

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**Figure 3** Mechanism of wound healing using SC-CM.
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