Acceleration of Wound Healing by Multiple Growth Factors and Cytokines Secreted from Multipotential Stromal Cells/Mesenchymal Stem Cells

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Background: Although multipotential stromal cells/mesenchymal stem cell (MSCs) initially gained attention because of their ability to differentiate into multiple cell lineages, it is their capacity to produce and secrete growth factors and cytokines that makes them particularly valuable as potential cell therapeutics.

The Problem: Wound healing is an intricate process consisting of several integrated stages, including angiogenesis, collagen production, and cell migration and proliferation. Coordinating these processes to ensure rapid and thorough wound healing is necessary when developing therapeutics. This coordination, however, is disrupted in chronic nonhealing wounds, wherein the impaired blood supply and resulting ischemia compromise cellular functions and make it difficult to deliver the necessary signaling molecules.

Basic/Clinical Science Advances: MSCs secrete a combination of growth factors and cytokines, which have been shown to promote wound repair. This combination of growth factors and cytokines successfully induces angiogenesis, reduces inflammation, and promotes fibroblast migration and collagen production.

Clinical Care Relevance: The growth factors and cytokines secreted by MSCs can be administered to wounds by either transplanting cells or, as a safer alternative, using the conditioned medium of MSCs, which contains these secreted bioactive molecules. For their success in reducing wound closure time, MSCs offer a promising option for treating chronic wounds. Still, possible undesirable effects of MSC-based therapeutics, such as keloid formation, need to be carefully studied.

Conclusion: With its strong ability to secrete diverse growth factors and cytokines, MSC-based therapeutics, either with cell transplantation or the conditioned medium, offers a novel approach toward chronic nonhealing wounds.

BACKGROUND

Multipotential stromal cells/mesenchymal stem cells (MSCs) are multipotent cells capable of differentiating into multiple cell lineages.¹ MSCs are relatively easy to obtain and expand *in vitro*.² In addition to autologous transplantation, allogenic MSC transplantation is also feasible, because these cells, like group O red blood cells, are immu-

nologically silent.³ These characteristics make MSCs good candidates for cell therapeutics against various diseases including chronic nonhealing wounds, against which there are no effective therapies available.

Initially, MSC differentiation and direct incorporation into regenerating tissues were speculated to be a primary mechanism of MSC action; however, the contribution of

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Abbreviations and Acronyms

bFGF = basic fibroblast growth factor

CXCL1 = chemokine ligand 1

HGF = hepatocyte growth factor

HIF = hypoxia inducible factor

IL-6/8 = interleukin-6/8

MAPK = mitogen-activated protein kinase

MSC = multipotential stromal cell/mesenchymal stem cell

MSC-CM = conditioned medium (media) of MSCs

pO₂ = partial oxygen pressure

VEGF = vascular endothelial growth factor

transdifferentiation and direct incorporation are rather controversial.¹ Rather, MSCs were shown to secrete various growth factors and cytokines such as vascular endothelial growth factor (VEGF), which promote angiogenesis and wound healing in host tissues.⁴ Some of the MSC's wound healing and tissue regenerative effects could, in fact, be reproduced by the conditioned medium of MSCs (MSC-CM), which include all of the bioactive molecules secreted by MSCs in culture.⁴

In this review article, we will digest the latest research articles about the role of MSC-derived growth factors and cytokines in MSC-based therapeutics in nonhealing chronic wounds.

TARGET ARTICLES

1. Yew TL, Hung YT, Li HY, Chen HW, Chen LL, Tsai KS, Chiou SH, Chao KC, Huang TF, Chen HL, and Hung SC: Enhancement of wound healing by human multipotent stromal cell conditioned medium: the paracrine factors and p38MAPK activation. *Cell Transplant* 2011; **20**: 693.

2. Lee EY, Xia Y, Kim WS, Kim MH, Kim TH, Kim KJ, Park BS, and Sung JH: Hypoxia-enhanced wound-healing function of adipose-derived stem cells: increase in stem cell proliferation and up-regulation of VEGF and bFGF. *Wound Repair Regen* 2009; **17**: 540.

CLINICAL PROBLEM ADDRESSED

Chronic nonhealing wounds represent a major burden in healthcare. More than 6 million people are estimated to be afflicted with chronic wounds, with a total estimated cost of more than \$25 billion annually in the United States.⁵ Chronic wounds cause serious consequences; for example, more than 70,000 lower limb amputations were performed on patients with diabetic foot problems in 2004 in the United States.⁵

Despite the recent advances in the medical science of wound healing, currently available therapeutics still attain only limited success against chronic wounds, and there is an urgent need for the development of novel effective therapeutics.⁶

RELEVANT BASIC SCIENCE CONTEXT

Wound repair is an indispensable ability for humans to sustain life. It is also one of the most complex biological processes involving multiple types of cells and bioactive molecules acting in an

orchestrated fashion. The normal wound healing process takes place in three distinct but overlapping stages—inflammation, new tissue formation, and remodeling⁷—and any arrests in these processes lead to the formation of nonhealing chronic wounds.

Vascular complications are often associated with chronic wounds. The resultant ischemia is one of the main contributing factors to the arrest of the wound healing process, as the limited supply of oxygen and other nutrients compromises cellular functions in injury sites and interferes with tissue repair processes.⁸ Therapeutic angiogenesis restores the blood supply to these ischemic wounds and promotes wound repair. Local administrations of angiogenic factors such as VEGF showed only limited success,⁹ suggesting that a combination of growth factors and cytokines rather than one specific growth factor is needed to attain neoangiogenesis with functional vasculatures. One possible solution is cytotherapy or cell transplantation with MSCs, as these cells are well known for secreting various growth factors and cytokines in response to environmental cues and can thus be expected to secrete these growth factors and cytokines in a coordinated manner.

Another common underlying condition causing nonhealing wounds is excessive inflammation. Although inflammation itself is essentially a body's protective response, persistent and excessive inflammation delays the wound repair process, especially in sterile or minimally contaminated wounds.¹⁰ Suppression of excessive inflammation should promote the repair process in chronic wounds. MSCs carry anti-inflammatory or immune-modulatory properties,³ and this might be one of the mechanisms through which MSCs promote wound healing.

In summary, MSC-based therapeutics is a promising approach to reverse the arrest of the wound repair process and promote the healing of chronic wounds.

EXPERIMENTAL MODEL OR MATERIAL: ADVANTAGES AND LIMITATIONS

To evaluate the roles of secreted growth factors and cytokines, MSCs were first cultured in the serum-free cell culture medium for 48–72 h and the “conditioned” medium was collected. The wound healing potency of these conditioned media was evaluated by an *in vitro* scratch assay as well as by a wound closure assay in mice models. The levels of growth factors and cytokines within the conditioned media were measured by enzyme-linked

immunosorbent assay. Neutralizing antibodies, chemical inhibitors, and siRNA were utilized to identify the molecules (growth factors, cytokines, and intracellular signaling molecules) pivotal for the accelerated wound healing mediated by MSC-CM.

Cell migration is a key underlying process during wound healing.⁷ *In vitro* scratch assay is a simple assay measuring the closure speed of the scratch wound artificially created on the monolayer of cultured cells. This system mimics the cell migration process during wound healing *in vivo* and is a widely used method to analyze cell migration *in vitro*. The main advantage of this assay is that it is simple and easy to perform but still provides a strong evaluation of the roles of crucial molecular events in wound healing.¹¹

On the other hand, the *in vitro* scratch assay is too simple to reproduce the entire *in vivo* wound healing process, as the wound healing process is an integration of several phases,⁷ and the *in vitro* scratch assay can only recapitulate a limited aspect of the wound healing process for a single cell type at a time.

DISCUSSION OF FINDINGS AND RELEVANT LITERATURE

Wounded tissues are hypoxic because of disruption of local vasculatures. The core of the wound is most hypoxic (partial oxygen pressure or pO_2 is 0–10 mmHg in dermal wound) with a progressive increase in the oxygen gradient toward the uninjured tissue at the periphery (pO_2 is 60 mmHg in dermal wound). This limited oxygen supply compromises cellular functions at injury sites, interfering with tissue repair processes such as angiogenesis and collagen synthesis.⁸ Thus, the wound hypoxia is a key limiting factor in wound healing and it is imperative to study the behavior of MSCs in a hypoxic condition.

Previous studies suggested that the hypoxic condition promotes self-renewal of undifferentiated MSCs and enhances therapeutic potential.^{2,12} Lee *et al.* showed that the MSC-CM collected in the hypoxic culture condition (hypoxia MSC-CM) induced higher type I collagen formation by fibroblasts, stronger fibroblast migration *in vitro*, and dermal wound

closure faster than MSC-CM collected in the normoxic culture condition.¹³ The hypoxia MSC-CM contained higher levels of growth factors including bFGF (basic fibroblast growth factor) and VEGF, and blocking antibodies against bFGF and VEGF reversed both fibroblast migration and wound closure induced by hypoxia MSC-CM, suggesting that these molecules play a crucial role in the MSC-CM-mediated wound healing process.

TAKE-HOME MESSAGE

Basic science advances

- MSCs secrete numerous growth factors and cytokines to accelerate wound healing and tissue regeneration.
- The hypoxic condition further increases the secretion of growth factors and cytokines from MSCs. These secreted molecules in turn stimulate fibroblasts in the wound areas to synthesize collagen and to migrate themselves, further accelerating wound repair process.
- Production of VEGF and bFGF by MSCs is upregulated in an HIF-dependent manner in the hypoxic condition.
- IL-6 also plays a pivotal role in MSC-mediated accelerated wound healing by promoting epithelial cell migration and angiogenesis. MSCs produce IL-6 in a p38MAPK pathway-dependent manner.
- Although IL-6 is one of the key mediators accelerating wound repair, excessive IL-6 secretion from MSCs might lead to keloid formation.

Clinical science advances

- MSCs are, like group O red blood cells, immunologically silent; thus, allogeneic MSC transplantation is also feasible, in addition to autologous MSC transplantation.
- MSC's secretion of paracrine factors, more than engraftment and differentiation, promotes the angiogenesis, anti-inflammatory effects, and cell migration necessary for wound repair.
- The condition medium of MSCs contains a combination of growth factors and cytokines that significantly accelerates wound repair and reduces wound closure time.
- The wound repair property of MSC-based therapeutics is superior to single growth factor administration alone.

Relevance to clinical care

- Chronic nonhealing wounds, which suffer from insufficient blood supplies and excessive inflammation, can benefit from the proangiogenic and anti-inflammatory effects of MSCs.
- MSC-based therapeutics offers a novel approach toward chronic nonhealing wounds.
- MSC condition medium might provide a safer alternative to living cells for delivering necessary growth factors and cytokines that work in conjunction to accelerate wound healing.
- Possible untoward effects of MSC-based therapeutics, such as keloid formation, need to be carefully evaluated.

Hypoxic exposure activates several signal transduction pathways including hypoxia inducible factor (HIF), a master transcription factor that regulates the expression of hundreds of genes to promote cellular adaptation to the hypoxic condition.¹⁴ Our latest study revealed that HIF-1 α is a pivotal signaling molecule for hypoxia-mediated upregulation of bFGF and hepatocyte growth factor (HGF) secretion, whereas HIF-2 α is a key signaling molecule for hypoxia-mediated upregulation of VEGF secretion from MSCs.² HIF-1 α is widely expressed in almost all tissues,¹⁴ whereas the expression of HIF-2 α is restricted to certain cell types such as vascular endothelial cells.¹⁵ MSC's proangiogenic trait is the most important property promoting the wound repair process, and VEGF is the key proangiogenic growth factor. Thus, HIF-2 α expression, in addition to ubiquitous HIF-1 α expression, should be crucial for MSC's wound healing property.²

MSCs secrete numerous growth factors and cytokines, and all the aspects of MSC's wound healing properties cannot be explained by VEGF, HGF, and bFGF alone. Among the molecules secreted from MSCs, Yew *et al.* found that interleukin-6 (IL-6) plays a major role in the MSC-mediated accelerated wound healing process by enhancing epithelial cell migration and inducing angiogenesis.¹⁶ IL-6 is a pleiotropic cytokine carrying both pro-inflammatory and anti-inflammatory properties¹⁷; indeed, IL-6 has been reported as one of the key molecules promoting the wound healing process through multiple mechanisms including leukocyte recruitment, angiogenesis, and collagen production.¹⁸ IL-6 is one of the molecules most highly secreted from MSCs, and it was shown to be a pivotal molecule involved in the MSC stemness *in vitro*.¹⁹ Thus, it is reasonable to expect that IL-6 would be one of the key molecules mediating the MSC-mediated wound healing property. In addition to IL-6, chemotactic cytokines (chemokines) IL-8 (IL-8) and chemokine ligand 1 (CXCL1) in MSC-CM were also shown to enhance epithelial cell migration in this study. Their data showed that the p38 mitogen-activated protein kinase (MAPK) pathway is a key intracellular signaling pathway regulating the secretion of these molecules from MSCs.

The target articles have a significant impact, because they demonstrated that MSC-CM, in addition to MSC itself, promotes the wound repair process and that multiple growth factors and cytokines secreted from MSCs, including VEGF, bFGF, IL-6, IL-8, and CXCL1, are involved in the MSC-mediated wound healing. These articles

clearly showed that MSC-CM can be regarded as a sort of pharmacological agent containing a mixture of various growth factors and cytokines promoting wound repair and tissue regeneration.

INNOVATION

The treatment of chronic nonhealing wounds is challenging, but accumulating evidence has suggested that MSC-based therapeutics is a promising option for this debilitating condition. As discussed earlier, MSC-based therapeutics has enormous potential for wound healing therapies.

Cell-based therapeutics is already clinically used for chronic nonhealing wounds, as exemplified by Dermagraft (Smith and Nephew/Advanced Tissue Sciences, London, United Kingdom), which includes living fibroblasts derived from neonatal foreskin, extracellular matrix, and a biodegradable scaffold.²⁰ MSCs carry even stronger paracrine activity,⁴ and thus, stronger wound healing effects are also anticipated.

Another innovative aspect of MSC-based therapeutics is that not only MSCs themselves but also acellular components such as MSC-CM carry strong therapeutic potentials. Acellular components are much easier for clinicians to handle than living cells when applying wound therapies. Another potential advantage of acellular components is safety. Malignant transformation during *in vitro* expansion or after transplantation is a potential concern for MSC-based therapeutics.²¹ Using acellular components for wound healing treatment could avoid this possibility.

SUMMARY ILLUSTRATION

The summary figure illustrates that transplanted MSCs enhance the wound repair process by stimulating proliferation and migration of keratinocytes and fibroblasts and inducing angiogenesis via secretion of growth factors and cytokines

such as VEGF, bFGF, HGF, and IL-6. MSC-CM containing these growth factors and cytokines carry similar wound healing properties as well.

CAUTION, CRITICAL REMARKS, AND RECOMMENDATIONS

Are there any possible adverse events with MSC-based therapeutics? Negative aspects of MSC-based therapeutics for wound repair have not been yet well addressed, but still, we can speculate based on the accumulating evidence from wound repair and MSC biology. As we discussed earlier, sustained elevation of inflammatory cytokines leads to deficient healing.²² Although MSCs have also been shown to exert anti-immunologic and anti-inflammatory effects,³ it is still possible that these MSC-derived cytokines might cause excessive inflammation through recruitment of more inflammatory cells and delay the wound healing process in certain situations. In addition, IL-6 has been shown to be involved in keloid formation, making keloid or excessive scar formation another possible unwanted effect of MSC treatment.²³ Further studies are needed to address the possible negative consequences of MSCs or MSC-CM.

FUTURE DEVELOPMENT OF INTEREST

MSC-CM contains a combination of growth factors and cytokines promoting wound repair; thus,

not only MSC itself but also MSC-CM could be used as a novel pharmacological agent for wound repair. However, MSC's paracrine activity declines during *in vitro* MSC expansion,²⁴ and interdonor variability of paracrine profile was also noted.²⁵ One possible solution is to choose the MSCs with the optimal paracrine profile and immortalize these cells in an early passage so that we could produce the ideal combination of growth factors and cytokines with minimum variability, possibly in a large scale in the GMP setting. It should also improve quality, because the levels of growth factors and cytokines could be easily monitored.

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The authors have no competing interests. This article was not written by any writer other than the authors.

REFERENCES

- Pittenger MF and Martin BJ: Mesenchymal stem cells and their potential as cardiac therapeutics. *Circ Res* 2004; **95**: 9.
- Tamama K, Kawasaki H, Kerpedjieva SS, Guan J, Ganju RK, and Sen CK: Differential roles of hypoxia inducible factor subunits in multipotential stromal cells under hypoxic condition. *J Cell Biochem* 2011; **112**: 804.
- Uccelli A, Moretta L, and Pistoia V: Mesenchymal stem cells in health and disease. *Nat Rev Immunol* 2008; **8**: 726.
- Caplan AI: Why are MSCs therapeutic? New data: new insight. *J Pathol* 2009; **217**: 218.
- Sen CK, Gordillo GM, Roy S, Kirsner R, Lambert L, Hunt TK, *et al.*: Human skin wounds: a major and snowballing threat to public health and the economy. *Wound Repair Regen* 2009; **17**: 763.
- Enoch S, Grey JE, and Harding KG: Recent advances and emerging treatments. *BMJ* 2006; **332**: 962.
- Gurtner GC, Werner S, Barrandon Y, and Longaker MT: Wound repair and regeneration. *Nature* 2008; **453**: 314.
- Sen CK: Wound healing essentials: let there be oxygen. *Wound Repair Regen* 2009; **17**: 1.
- Dvorak HF: Angiogenesis: update 2005. *J Thromb Haemost* 2005; **3**: 1835.
- Szpaderska AM and DiPietro LA: Inflammation in surgical wound healing: friend or foe? *Surgery* 2005; **137**: 571.
- Liang CC, Park AY, and Guan JL: *In vitro* scratch assay: a convenient and inexpensive method for analysis of cell migration *in vitro*. *Nat Protoc* 2007; **2**: 329.
- Grayson WL, Zhao F, Izadpanah R, Bunnell B, and Ma T: Effects of hypoxia on human mesenchymal stem cell expansion and plasticity in 3D constructs. *J Cell Physiol* 2006; **207**: 331.
- Lee EY, Xia Y, Kim WS, Kim MH, Kim TH, Kim KJ, *et al.*: Hypoxia-enhanced wound-healing function of adipose-derived stem cells: increase in stem cell proliferation and up-regulation of VEGF and bFGF. *Wound Repair Regen* 2009; **17**: 540.
- Semenza GL: Oxygen-dependent regulation of mitochondrial respiration by hypoxia-inducible factor 1. *Biochem J* 2007; **405**: 1.
- Tian H, McKnight SL, and Russell DW: Endothelial PAS domain protein 1 (EPAS1), a transcription factor selectively expressed in endothelial cells. *Genes Dev* 1997; **11**: 72.
- Yew TL, Hung YT, Li HY, Chen HW, Chen LL, Tsai KS, *et al.*: Enhancement of wound healing by human multipotent stromal cell conditioned medium: the paracrine factors and p38MAPK activation. *Cell Transplant* 2011; **20**: 693.
- Jawa RS, Anillo S, Huntoon K, Baumann H, and Kulaylat M: Analytic review: interleukin-6 in surgery, trauma, and critical care: part I: basic science. *J Intensive Care Med* 2011; **26**: 3.
- Lin ZQ, Kondo T, Ishida Y, Takayasu T, and Mukaida N: Essential involvement of IL-6 in the skin

- wound-healing process as evidenced by delayed wound healing in IL-6-deficient mice. *J Leukoc Biol* 2003; **73**: 713.
19. Pricola KL, Kuhn NZ, Haleem-Smith H, Song Y, and Tuan RS: Interleukin-6 maintains bone marrow-derived mesenchymal stem cell stemness by an ERK1/2-dependent mechanism. *J Cell Biochem* 2009; **108**: 577.
20. Jimenez PA and Jimenez SE: Tissue and cellular approaches to wound repair. *Am J Surg* 2004; **187**: 56S.
21. Lepperdinger G, Brunauer R, Jamnig A, Laschober G, and Kassem M: Controversial issue: is it safe to employ mesenchymal stem cells in cell-based therapies? *Exp Gerontol* 2008; **43**: 1018.
22. Henry G and Garner WL: Inflammatory mediators in wound healing. *Surg Clin North Am* 2003; **83**: 483.
23. Ghazizadeh M, Tosa M, Shimizu H, Hyakusoku H, and Kawanami O: Functional implications of the IL-6 signaling pathway in keloid pathogenesis. *J Invest Dermatol* 2007; **127**: 98.
24. Crisostomo PR, Wang M, Wairiuko GM, Morrell ED, Terrell AM, Seshadri P, *et al.*: High passage number of stem cells adversely affects stem cell activation and myocardial protection. *Shock* 2006; **26**: 575.
25. Zhukareva V, Obrocka M, Houle JD, Fischer I, and Neuhuber B: Secretion profile of human bone marrow stromal cells: donor variability and response to inflammatory stimuli. *Cytokine* 2010; **50**: 317.