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Developing Mesenchymal Stem Cell-Based Therapy for Severe Skin Burn Wounds

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DRDC Suffield

Defence R&D Canada – Suffield
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Technical Memorandum
DRDC Suffield TM 2012-028
April 2012

Canada

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Abstract

One of the most common battlefield afflictions is severe skin injury, including burn trauma. Although medical strategies have been developed to improve burn wound healing, current approaches still fall short of restoring normal appearance and function in the case of third-degree (full-thickness) wounds. In the normal healing process, skin wounds do not regenerate the damaged tissue, but rather replace it with scar tissue which lacks normal function and appendages such as hair follicles and subcutaneous glands. The formation of fibrotic scars after wounding poses a major medical problem and expense. Skin scarring and appendage deficiency, following wound healing, often result in loss of skin function, restriction of mobility leading to disability, and adverse psychological effects, all of which severely affect the quality of life for patients. Application of mesenchymal stem cells (MSCs) to excision and burn wounds has been shown to improve the rate and integrity of the natural healing process. Mounting evidence suggests that transplanted MSCs recruit endogenous stem cells to the site of injury and attenuate the inflammatory response of the host through a paracrine (localized) response. Importantly, human MSCs are immune-privileged, and thus can be transplanted between individuals without rejection. However, current MSC-based therapies do not produce seamless skin regeneration, suggesting that the innate ability of MSCs to improve wound healing is limited. The ability of therapeutic MSCs to regenerate functional tissue might be improved through genetic modification, in which MSCs are engineered to deliver factors known to minimize scar formation and promote skin regeneration. In this manner, the innate healing power of applied MSCs can be coupled with gene therapy to fully realize the possibility of seamless skin wound repair and regeneration.

Résumé

Les lésions cutanées graves, dont les brûlures, comptent parmi les affections les plus courantes sur le champ de bataille. Malgré la mise au point de stratégies médicales en vue d'améliorer la cicatrisation des brûlures, les méthodes actuelles ne permettent toujours pas de rétablir l'apparence et la fonction normales à la suite de lésions au troisième degré (profondes). Au cours du processus normal de cicatrisation, les tissus endommagés ne sont pas régénérés, mais plutôt remplacés par du tissu cicatriciel. Ce dernier est dépourvu de phanères, comme les follicules pileux et les glandes sous-cutanées, et il s'ensuit une perte fonctionnelle. De plus, la formation de cicatrices fibreuses après une blessure représente un problème médical important et coûteux. Une fois la lésion guérie, les cicatrices et la disparition des phanères entraînent souvent une perte de fonction de la peau, une restriction de la mobilité à l'origine d'incapacités et des effets psychologiques indésirables. Tous ces facteurs peuvent affecter grandement la qualité de vie des patients. L'application de cellules souches mésenchymateuses (CSM) sur des plaies par excision ou par brûlure a permis d'améliorer la vitesse et l'intégrité du processus naturel de cicatrisation. Des données de plus en plus nombreuses indiquent que les CSM transplantées recrutent des cellules souches endogènes dans la région lésée et atténuent la réaction inflammatoire de l'hôte par un effet paracrine (local). Fait important, grâce au privilège immun, la transplantation de CSM humaines entre deux individus n'entraîne pas de rejet. Cependant, les traitements actuels à base de CSM ne permettent pas une régénération cutanée homogène, ce qui donne à penser les CSM ont une capacité intrinsèque limitée d'améliorer la cicatrisation des plaies. La capacité des CSM thérapeutiques à régénérer un tissu fonctionnel pourrait être intensifiée par modification génétique, de sorte que les CSM libèrent des facteurs qui limitent la formation de cicatrices et qui favorisent la régénération cutanée. Ainsi, le pouvoir de guérison intrinsèque des CSM appliquées pourrait être combiné à la thérapie génique pour rendre possibles une réparation et une régénération optimales des plaies cutanées.

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Executive summary

Developing Mesenchymal Stem Cell-Based Therapy for Severe Skin Burn Wounds

W-G. Hu; L.R. Braid; and L.P. Nagata; DRDC Suffield TM 2012-028; Defence R&D Canada – Suffield; April 2012.

Introduction: Massive skin trauma is a common event in the military environment. War-related skin injury has a long, global history, and in modern warfare, one in four skin injuries is a burn. Although current medical strategies have improved critical care to save more lives of morbidly injured patients than in previous wars, these commonly produce excessive scars which lack both form and function. Pathological scarring produces non-functional tissue at the wound site, leading to skin dysfunction, deformities, and restricted mobility leading to disability. Patients often suffer from psychological trauma as a consequence of scar-induced disfigurement and disability. The financial burden of cosmetic surgery, physical and psychiatric rehabilitation to treat patients with pathological scarring is staggering. As such, current medical research has a focus on skin regeneration. The aim is to re-direct the physiological wound healing response from the deposition of non-functional tissue (scars) to a process that regenerates functional skin structure, including all epidermal appendages like hair follicles, sweat glands, and sebaceous glands. Stem cells are emerging as a very promising approach for tissue and organ regeneration. In the near future, stem cell-based therapy for regenerative medicine could be the breakthrough equivalent to that of antibiotics in the last century.

Results: In this technical memorandum, an overview of the most recent advances and future perspectives for severe skin wound healing using mesenchymal stem cells (MSCs) is presented. Although many research studies have demonstrated that transplanted MSCs can improve wound healing, these have also revealed that MSCs do not eliminate scarring and rarely differentiate into epithelial cell lineages, especially skin appendage cell types. The limited capacity of MSCs to regenerate epithelial cell types might be improved through a genetic modification approach, in which MSCs are engineered to either direct them to acquire an epithelial identity, or to secrete factors that attract the host's epithelial stem cells to re-populate the wound site. In addition, therapeutic MSCs can be engineered to deliver factors known to reduce inflammation, protect against infection and minimize scarring. Genetically modified MSC therapy, which couples the innate healing power of applied MSCs with the targeted delivery of factors known to minimize scar formation and promote skin regeneration, holds great promise for full regeneration of severe skin wounds.

Significance: The results of this survey will assist in the development of genetically modified MSC-based therapy for severe skin burn wound healing by DRDC Suffield.

Future plans: The genetically modified MSCs suggested in this memorandum will be developed and tested in order to develop an effective therapeutic approach to improve severe skin burn wound healing. Genetic engineering of MSCs to express specific genes encoding growth factors or cytokines would allow the MSCs to deliver sustained, therapeutic levels of regenerative factors, thereby enhancing the capacity of MSCs to improve wound healing. The expected physiological outcomes of MSC-mediate gene therapy include reducing inflammation to

accelerate healing and minimize scarring, and the recruitment and activation of endogenous stem cells from uninjured skin to the wound to regenerate normal skin architecture, composition and function.

Sommaire

Élaboration d'un traitement à base de cellules souches mésenchymateuses pour les brûlures graves

W-G. Hu; L.R. Braid; et L.P. Nagata; RDDC Suffield TM 2012-028; R & D pour la défense Canada – Suffield; avril 2012.

Introduction : Les traumatismes cutanés importants sont fréquents dans l'environnement militaire. Depuis longtemps à l'échelle mondiale, les lésions cutanées font partie des blessures liées à la guerre, et dans le contexte de la guerre moderne, une lésion cutanée sur quatre est une brûlure. Grâce au progrès des soins aux malades en phase critique, il est maintenant possible de sauver la vie d'un plus grand nombre de blessés qu'au cours des guerres passées. Néanmoins, les stratégies médicales actuelles entraînent fréquemment l'apparition de cicatrices excessives, qui sont déficientes sur le plan morphologique et fonctionnel. En cas de cicatrisation pathologique, des tissus non fonctionnels se forment au siège de la lésion, ce qui engendre un dysfonctionnement cutané, des déformations et une restriction de la mobilité à l'origine d'incapacités. Les patients subissent souvent un traumatisme psychologique en raison du préjudice esthétique et de l'incapacité attribuables aux cicatrices. Par ailleurs, les soins de chirurgie esthétique et la réadaptation physique et psychiatrique des patients qui présentent des cicatrices pathologiques engendrent un fardeau financier incroyablement élevé. Dans ce contexte, la recherche médicale actuelle est axée sur la régénération cutanée. L'objectif consiste à modifier le processus physiologique de cicatrisation pour empêcher l'apparition de tissus non fonctionnels (cicatrices) et rétablir l'intégrité fonctionnelle de la peau, y compris l'ensemble des phanères, comme les follicules pileux, les glandes sudoripares et les glandes sébacées. Les cellules souches représentent une nouvelle approche très prometteuse pour la régénération des tissus et des organes. Dans un avenir rapproché, la mise au point de traitements à base de cellules souches en médecine régénérative pourrait constituer une percée comparable à l'avènement des antibiotiques au siècle dernier.

Résultats : Le présent document technique trace un aperçu des avancées les plus récentes et des perspectives d'avenir en ce qui a trait à l'utilisation de cellules souches mésenchymateuses (CSM) dans le traitement des plaies cutanées graves. Si de nombreuses études ont montré que la greffe de CSM peut favoriser la guérison, ces études ont aussi révélé que les CSM n'éliminaient pas la formation de cicatrices et se différenciaient rarement en lignées de cellules épithéliales, en particulier celles correspondant aux phanères. La capacité restreinte des CSM à régénérer les différents types de cellules épithéliales pourrait être intensifiée par modification génétique, par la création de CSM conçues pour acquérir une identité épithéliale ou pour sécréter des facteurs qui attireront les cellules souches épithéliales de l'hôte, afin que ces dernières réintègrent la zone lésée. En outre, les CSM thérapeutiques peuvent être conçues de façon à ce qu'elles libèrent des facteurs connus pour réduire l'inflammation, prévenir l'infection et limiter la formation de cicatrices. Le traitement par CSM génétiquement

modifiées s'avère très prometteur pour la régénération complète de la peau gravement lésée, en associant le pouvoir de guérison intrinsèque des CSM appliquées et la libération ciblée de facteurs connus pour limiter la formation de cicatrices et promouvoir la régénération de la peau.

Importance : Les résultats de cette étude aideront RDDC Suffield à mettre au point un traitement à base de CSM génétiquement modifiées pour la guérison des brûlures cutanées graves.

Plans futurs : Les CSM génétiquement modifiées proposées dans le présent document seront élaborées et mises à l'essai dans l'objectif de mettre au point une méthode thérapeutique efficace pour améliorer la cicatrisation des brûlures cutanées graves. La modification des CSM par génie génétique, en vue de l'expression de gènes spécifiques qui codent pour des facteurs de croissance ou des cytokines, permettrait aux CSM de libérer des facteurs régénératifs à des niveaux thérapeutiques soutenus. Les CSM auraient ainsi une capacité supérieure de favoriser la guérison. Les résultats physiologiques attendus d'une thérapie génique médiée par les CSM comprennent, d'une part, la réduction de l'inflammation en vue d'accélérer la guérison et de réduire la formation de cicatrices et, d'autre part, le recrutement et l'activation des cellules souches endogènes de la peau saine pour rétablir l'architecture, la composition et la fonction normales de la peau lésée.

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1 Introduction

Massive skin trauma is a common event in the military environment. In modern warfare, one in four skin injuries involves a burn, while burn injuries account for 5% to 10% of total combat casualties [1, 2]. Nearly 20% of combat burn injuries are categorized as severe (involving more than 20% of total body surface area (TBSA)) and require significant resuscitation [2]. Such injuries have increased with the evolution of more sophisticated explosive devices in recent conflicts [3] — the conflict in Iraq resulted in proportionately far more severely burned soldiers than previous wars [4–6]. US military severe burn casualties are evacuated by aircraft staffed with specially trained medical personnel to Brooke Army Medical Institute in San Antonio, Texas, a dedicated burn treatment and rehabilitation centre [7]. Canadian Armed Forces (CAF) burn casualties are treated at the civilian burn centre closest to their hometown. However, regardless of where treatment occurs, although current medical strategies have improved critical care to save more lives of morbidly injured patients than in previous wars [8], excessive scars which lack both structure and function commonly result [2, 9, 10]. Pathological scarring produces non-functional tissue at the wound site, leading to skin dysfunction, deformities, and restricted mobility, leading to disability [9, 11, 12]. In addition, casualties often suffer psychological trauma as a consequence of scar-induced disfigurement and disability [13, 14].

1.1 Skin wound healing and scar formation

The skin is the largest organ of the body and functions as a protective barrier against a hostile environment. It not only plays an active role in the immune system, it is also involved in maintaining the optimal temperature for the body to function and gathers sensory information from the environment.

The skin consists of three distinct tissue layers: the epidermis, the dermis, and subcutaneous tissue (Figure 1) [15]. The epidermis, which derives from the embryological ectoderm, is the outer layer of skin and consists of multiple layers of keratinocytes that form a stratified squamous epithelium. The basement layer has cells that are shaped like columns. In this layer the cells divide and push already-formed cells into higher layers. As the cells move into the higher layers, they flatten and eventually die. The epidermis acts as a barrier against exogenous substances and pathogens, as well as water loss. The next layer, the dermis, which derives from the embryological mesoderm, is composed of fibroblasts that synthesize extracellular matrix, endothelial cells in small vessel capillaries, and appendages. Skin appendages, including hair follicles, sweat glands and sebaceous glands, are derived from the epidermis but are buried in the network of collagen and elastin fibers in the dermal layer. The functional properties of the dermis include support and nourishment to the epidermis, mechanical strength and elasticity to the skin, regulation of body temperature via hair follicles, sweat glands and dermal capillaries, and lubrication of the epidermis via sebaceous glands. These dermal properties are often under-appreciated until substantial loss of the skin occurs.

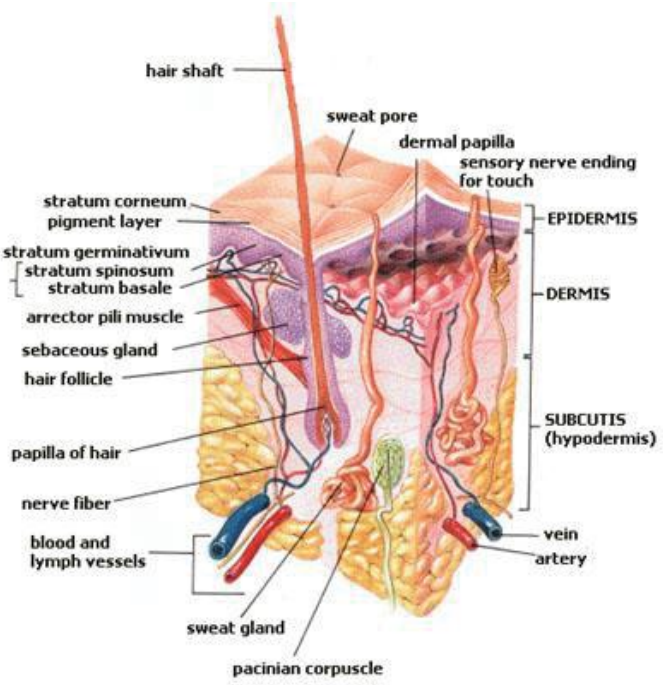

Figure 1: Structure of the skin.

(Image is in the public domain.)

The subcutaneous tissue layer (or hypodermis), which also derives from the embryological mesoderm, lies below the dermis and consists of fat tissue, which helps insulate the body from heat and cold, provides protective padding, and serves as an energy storage area. The fat is contained in living adipose cells, which are supported by a fibrous network.

Severe skin injury, including burns, results in loss of the epidermis with additional damage extending to the dermis, or in the case of third-degree burns, even deeper to the subcutaneous tissue. Tissue disruption in humans does not result in tissue regeneration, but in a rapid repair process leading to a fibrotic scar. Wound healing is a multifaceted process orchestrated by numerous cell types and a complex interplay of signals emanating from the damaged cells and mediators of the immune response. Injury to the skin initiates a cascade of events including clot formation, cell migration, extracellular matrix synthesis and deposition, and finally, dermal and epidermal reconstitution and re-modeling. The healing process can be divided into three chronological phases, inflammation, proliferation, and maturation (or re-modeling) [9, 16–18].

In the first phase, inflammatory cells invade the wound tissue in response to damage signals produced by ruptured cells and blood vessels. Neutrophils arrive first. These mediate the primary defence against microorganisms, and stimulate phagocytosis of cell debris. Most importantly, they secrete growth factors and cytokines which attract migrating progenitor cells and initiate the next phase. In the second phase, granulation tissue forms a temporary wound covering and is the product of three essential steps:

- Proliferation of endothelial cells results in massive angiogenesis, the formation of new blood vessels.
- Proliferation of fibroblasts contributes to collagen production and deposition. The synthesis of extracellular matrix (ECM) combined with neo-vascularisation re-establishes connective tissue in the wound bed. This newly-formed support matrix provides the essential scaffold, nutrients and oxygen to progenitor cells migrating into the wound.
- Proliferation of keratinocytes, which have infiltrated the wound from neighbouring undamaged tissue, results in re-epithelialisation leading to wound closure.

During maturation or re-modeling, resolution of wound healing is marked by the elimination of inflammatory cells, apoptosis of supernumerary progenitor cells, and re-modeling of the extracellular matrix and vascular networks. In the second phase of wound healing, ECM and the vascular networks are over-produced and haphazardly deposited in an effort to rapidly seal the wound. These effects necessitate a prolonged re-modeling phase to cull the excess network and re-establish it in the refined architecture typical of normal skin. Unfortunately, the abnormal deposition of granulation tissue can never be completely replaced and the wound is permanently sealed by scar tissue. Scarring is caused by over-deposition of interstitial collagens accompanied by excessive proliferation of fibroblasts. A high rate of collagen synthesis generally lasts several months post-wounding and matrix re-modeling continues for many months. Although scar tissue restores tissue integrity, it remains weaker than normal skin and lacks skin appendages.

1.1.1 Severe burn injuries

Burns are classified by the depth of tissue damage (degree) and the affected TBSA [19]. First-degree burns are the mildest burn injury, affecting only the superficial epidermis resulting in redness without blistering. Second-degree, or partial-thickness, burns affect the epidermis and dermis, and are classified as either superficial (damage into the papillary dermis) or deep (extend into the reticular dermis) [19]. These burns are characterized by moderate to severe blistering, and only require attention from burn specialists in the case of deep partial-thickness wounds or injuries sustained by complex organs such as the face, hands, joints or genitalia. Superficial wounds will fully heal in two to four weeks with minimal or no scarring, while mid- to deep-thickness wounds require eight weeks or longer, and the aid of skin grafts or skin substitutes, to heal with a range of skin defects from discoloration to moderate scarring at the injured site.

Third-degree, or full-thickness, burns produce a significant amount of dead or necrotic tissue extending to the subcutaneous tissue [19]. While first- and second-degree burns only cause cellular damage, the epidermis and dermis are utterly destroyed by third-degree burns. The consequent death of blood vessels at the interface of the dermal and subcutaneous layers impedes blood-flow to the wound site; since the body`s primary immune defence components are delivered by the vascular system, these wounds are susceptible to infection. There are no residual epidermal progenitor cells to repopulate the area, so wound closure can only be achieved by skin grafts after the necrotic tissue has been surgically removed (escharotomy). Third-degree burns covering more than 20% TBSA are associated with a high degree of mortality [20, 21]. Recovery from extensive fourth-degree burns, which damage the subcutaneous layer and often the underlying muscle, fascia, or bone, is rare.

An incident severe enough to cause third- or fourth- degree burns often affects a greater surface area of the body than milder burns. Debridement of the wound and closure by grafting is imperative at the earliest opportunity after the patient is stabilized, in order to prevent systemic inflammatory response syndrome (SIRS), infection, and wound conversion [19]. SIRS is a subset of a cytokine storm, in which an unregulated inflammatory response induces sepsis and can result in death [19]. Wound conversion is a dynamic and destructive process in which injured cells at the periphery of the wound die, which expands the central necrotic zone. As such, untreated burn wounds grow, increasing in severity and complexity, further reducing blood flow and increasing the risk of infection and debilitating scar formation [19]. In current surgical theatres, various skin substitutes are used to close the wound when donor grafts from the patient are not sufficient. Skin grafts can be obtained from cadavers (human allografts), pigs (xenografts), and human amniotic membrane [22]. Alternatively, biosynthetic skin substitutes are increasingly common surgical tools. The combination of technological advancements and a more comprehensive knowledge of skin biology are improving the sophistication of these products to more closely recapitulate the physiology and function of normal human skin. Examples of such products include acellular matrices derived from the submucosa of the porcine small intestine and silicone bilayer membranes loaded with human keratinocytes or neonatal foreskin fibroblasts [23]. Other cutting-edge technologies use the limited graft tissue from the patient to harvest epithelial stem cells, which are cultured in a lab and re-applied to the patient in an array of formats such as thin epithelial sheets [24] or spray-gun coating [25, 26]. This *ex vivo* expansion of autologous stem cells before transplant (cultured epithelial autograft) multiplies the cells up to ten-thousand times the size of the original biopsy site, but requires two to three weeks [27, 28]. The standard use of antimicrobial silver-treated dressings aids in preventing infection before and after graft surgery [22].

1.2 Purpose

Although medical strategies have been developed that improve burn wound healing, current approaches still fall short of restoring normal appearance and function in the case of third-degree (full-thickness) wounds. To address this deficiency, there has been widespread medical research focussed on skin regeneration, the aim of which has been to re-direct the physiological wound healing response from the deposition of non-functional tissue (scars) to a process that regenerates functional skin structure, including all epidermal appendages like hair follicles, sweat glands, and sebaceous glands, and which restores all aspects of skin function [29–31].

This technical memorandum presents an overview of the most recent advances in the field of skin wound healing, with an emphasis on burn injury, using mesenchymal stem cells (MSCs).

2 Overview of recent research

2.1 Healing differences between fetal and adult skin wounds

Fetal wound repair is fundamentally different from adult repair. In adult tissues, the physiological response is acute inflammation and fibrosis, in which collagen fibres are rapidly and haphazardly deposited in an effort to seal the wound [9, 16–18]. The fibrotic response produces scar tissue at the expense of regenerating the damaged tissue. The response to wounding is markedly different in the developing fetus, where the absence of inflammation permits seamless regeneration of injured tissue, and the complete absence of scarring [32–34]. Until the third trimester, the fetus can fully restore epidermal and dermal structure — including extracellular matrix architecture and tensile strength — and function [35–38]. As such, a major goal of regenerative research is to characterize the cellular and molecular differences between scar-less and fibrotic wound healing, in order to identify new therapeutic targets that will recapitulate the fetal healing process in adults.

In fetal wounds, collagen is rapidly deposited in a fine reticular pattern, while in adult wounds, the collagen bundles are packed tightly together in large parallel fibers, unlike the reticular pattern in unwounded skin [32–34, 39]. One of the defining characteristics of scar-less repair is a relative lack of inflammation [40–44]. Indeed, exogenous stimulation of inflammation in fetal wounds results in dose-dependent increases of macrophages, neutrophils, fibroblasts, collagen deposition, and finally scarring [45]. Moreover, it is suggested that the infiltration of inflammatory cells likely disrupts the inherent ability of resident dermal cells to restore the dermal structure [45]. Studies such as this reveal the critical role of inflammation in scar formation, and suggest that attenuating inflammation in adult wounds can promote scar-less wound healing.

Adult tissues and organs also have a limited regenerative capacity compared to the developing fetus. Adult responses to wounding do not readily regenerate the components of normal skin architecture. It is speculated that fibroblasts are the primary cell type responsible for the production of scar-less versus fibrotic tissue [32, 33, 46]. This was demonstrated in a pivotal study, where transplantation of fetal fibroblasts to an adult environment accomplished scar-less wound healing [47]. During the proliferative phase of wound healing, fibroblasts rapidly proliferate to efficiently seal the wound. Fetal fibroblasts deposit matrix in an organized fashion resembling ECM in normal skin, which lays the groundwork for stereotypical neo-vascularisation and epidermal proliferation and differentiation from the outset [32, 33, 46]. The abnormal deposition of collagen by adult fibroblasts has a detrimental effect, as the synthesis of an atypical scaffold misdirects the formation of vascular networks, culminating in fibrotic scar tissue [32, 33, 46, 48]. Important differences in the gene expression profiles of fetal and adult dermal fibroblasts suggest that therapeutic approaches could be developed to manipulate these progenitor cells to elicit a fetal-type response during adult wound healing [32, 33, 46]. A recent study demonstrated the viability of such an approach, where gene transfer was used to exogenously express a transcription factor, Ski, in wound bed fibroblasts [49]. Expression of Ski simultaneously drove fibroblast proliferation and inhibited collagen secretion, resulting in accelerated wound healing, reduced inflammation, accelerated re-epithelialisation, and reduced scar formation in rat skin and rabbit ear excision wounds [49]. There is mounting experimental evidence to support the notion

that therapies which suppress inflammation and modify fibroblast behaviour have the potential to recapitulate the fetal, scar-less response to wound healing in adult tissue.

2.2 Stem cell-based therapy

Stem cells are distinguished from other cell types by two important characteristics [31, 50]. First, they are unspecialized cells capable of renewing themselves through cell division, sometimes after long periods of inactivity. Second, their progeny can undergo rapid proliferation and be induced to become tissue- or organ-specific cells with specialized functions under certain physiological or experimental conditions. Consequently, a minimal number of stem cells can seed the re-population of a wound, generating most, if not all, of the cell types that constitute the damaged tissue, organ or appendage.

Excitingly, recent studies have shown that stem cells can be induced to differentiate into various cell types with specialized functions and can participate in tissue and organ regeneration in almost all lesions [23, 29–31, 51–54]. There is also mounting evidence that transplanted stem cells secrete factors that stimulate a regenerative response from the wounded patient [55–58]. Current evaluations of stem cell therapies for severe excision and burn wound healing have consistently demonstrated acceleration of wound closure with reduced scarring and improved function compared to controls [23, 57, 59–63]. Thus, stem cell-based therapies are a promising approach for improving severe skin wound healing.

The two main sources of stem cells that can be used for repair and regeneration of injured tissues are embryonic and post-natal/adult [31]. Each stem cell origin has unique benefits and drawbacks, which must be considered when choosing the type of stem cells for a given application [29, 31, 50, 64]. Embryonic stem cells have the greatest differentiation potential, as they are pluripotent and can be induced to adopt any type of somatic cell lineage. However, this developmental plasticity also makes them prone to tumorigenesis following transplant. Embryonic stem cells are also technically challenging to culture as they require the co-culture of specialized feeder cells, daily replacement of nutrients, and are notoriously prone to spontaneous differentiation and senescence. The clinical applications of embryonic stem cells are also limited because of political and ethical considerations. The use of post-natal or adult stem cells circumvents the political, ethical, and tumorigenic problems associated with pre-natal stem cells, but their developmental potential is comparatively limited. Adult-derived stem cells are multipotent and are typically committed to producing cell types limited to the lineage specified by the embryological germ layer (mesoderm, ectoderm, or endoderm) from which they originate.

Adult stem cells have been successfully isolated from many tissues and organs, including bone marrow, blood, adipose tissue, intestine, hair follicle, liver, umbilical cord blood and tissue, and neurons [31, 50]. The primary role of adult stem cells in a living organism is to maintain and repair the tissue in which they reside. Typically, a very small number of stem cells reside in specialized niches in each tissue, where they divide infrequently and can exist for many years [50, 65]. The niche provides a physically protective environment, as well as the signalling milieu required to maintain stem cell survival and to provide proliferation and differentiation cues. Once stem cells are extracted from the niche, their capacity to survive and to divide is greatly limited, making the production of large quantities of undifferentiated stem cells *ex vivo* difficult [65].

A considerable body of evidence indicates that the most suitable adult stem cells for use in therapeutic applications are mesenchymal stem cells (MSCs) as [62, 66, 67]. They comprise a heterogeneous family of plastic-adherent stem cells which can be isolated from the stroma (connective tissue) from a variety of sources, including bone marrow, skeletal muscle, adipose tissue, blood, umbilical cord, and amniotic fluid [31, 55, 69–75]. MSCs differentiate mainly towards mesodermal-derived cell types — osteoblasts (bone), chondroblasts (cartilage), and adipocytes (fat) [55, 76–79]. Considerable variability in the proliferative and differentiation potential of MSCs, even within a single cell population, has been reported [31, 80–82]. Although an active search for definitive stem cell markers continues in the field at large, it is generally accepted that MSC populations constitute a mix of cell subtypes that meet the minimal criteria for defining stem cells, but also display distinct characteristics, as well as corresponding markers, that confer different developmental potentials. The minimal defining criteria for MSC populations were laid out by the International Society for Cellular Therapy in 2006 [83]:

- The cells adhere to plastic under standard culture conditions;
- Most of them (>95%) express CD73 (5′-ectonucleotidase), CD90 (Thy-1) and CD105 (endoglin);
- At least 98% of them do not express haemoatopoetic markers CD45, CD34, and CD14; and
- They can differentiate *in vitro* along the osteoblastic, chondrogenic, and adipogenic lineages.

Compared to other adult stem cells, heterogeneous MSC populations exhibit a greater potential to differentiate into more embryologically distant cell types, including epithelial cell lineages [83], or even skin appendages [84]. They also communicate with a broader array of cell types, and can integrate into various types of stem cell niches [55]. These characteristics make them highly amenable for broad use therapies in wound healing. Importantly, MSCs are one of the few adult stem cells that can be isolated and that can proliferate robustly and without differentiation *ex vivo* to produce adequate quantities for therapeutic applications [86]. These attributes, conferred by the heterogeneity of stem cell subtypes in an MSC population, make MSCs highly attractive for transplantation and wound healing applications.

Recent studies have shown that transplanted MSCs accelerate wound healing in both animal models and humans [74, 86–89]. In Russia, the first human study using MSCs for burn wound therapy was conducted on a female patient with extensive burns (I-II-IIIAB skin burn, total area 40%, area of IIIB degree 30%) [88]. MSCs harvested from the patient's bone marrow were transplanted at the wound site, resulting in rapid healing of the wound and accelerated rehabilitation. Stem cell therapies have historically required this type of allogenic transfer, in which stem cells are harvested from the patient, potentially modified for therapy, and then re-introduced at the site of injury. However, this process requires invasive and painful surgical procedures on the already-injured patient and introduces delays in initiating treatment, both of which are detrimental to the healing process.

How transplanted MSCs contribute to the wound healing process is not yet fully understood, but current research is revealing an array of mechanisms. Studies have demonstrated that transplanted MSCs can migrate to injured tissues in response to various chemokines [56, 57, 90]; they then secrete growth factors and chemokines to stimulate paracrine effect on cells surrounding the injured site [56, 57]. These signals can modulate the immune and inflammatory responses [91]

and attract endogenous stem cells to the wound site [57]. In addition to their signalling roles, MSCs physically contribute to wound closure and regeneration. In wound healing assays, MSCs can differentiate into mesenchymal cell lineages (such as endothelial cells, adipocytes, and stromal fibroblasts) to re-populate the wound site [57, 92, 93]. MSCs have also been shown to manufacture extracellular matrix, including collagen and fibronectin proteins [94], which generates the structural framework necessary for wound closure and patterning the regenerating tissue. Through these combinatorial effects, MSCs can enhance the recruitment of cells to the wound [57], stimulate angiogenesis [92, 95], and improve survival and function of endogenous cells at the site of injury, resulting in accelerated and improved wound closure [96].

MSCs are particularly attractive for transplant applications because they do not express many of the cell surface receptors recognized by the immune system [66, 674, 85]. This enables them to escape detection by the host's immune system and circumvents graft-versus-host rejection, one of the greatest barriers to stem cell transplant therapies. Thus, immune-privileged MSCs hold great promise for the pre-production of cell therapies that can be introduced to any human patient without rejection. Such immune-privileged MSCs have been successfully transplanted between individuals of the same species (allogenic transfer) [97–99] and, in rare instances, between different organisms (xenogenic transfer) [89, 100].

The ultimate goal for MSC-based therapies in the treatment of severe skin wounds is to seamlessly regenerate all the structural components and functions of native skin. Although MSCs appear to be the most suitable type of stem cell for wound healing applications, they have one significant limitation for regenerating massive skin injuries. MSCs originate from the mesoderm, and consequently have very little capacity to adopt an epithelial fate and differentiate as epidermis and skin appendages. To date, there is no source of adult MSC that can replicate the complexity of cell types that comprise human skin. In addition, scar-less wound healing has not yet been achieved using MSC therapy.

2.3 Therapeutic approaches employing cytokines and other regulatory biomolecules

Administration of a number of cytokines and other regulatory biomolecules to skin wounds has been shown to positively influence wound healing. While direct administration of such proteins into wounds has been shown to have positive effects, these are necessarily limited, since proteins are typically subject to rapid degradation. In addition, direct administration as a method of therapy, and not just as a research tool, would require the production and purification of large quantities of protein *in vitro*. A further complication for direct administration is that many proteins require post-translational processing in order to be functional. If these enzymatic processes fail to occur *in vitro*, then the manufactured proteins will be inactive and unusable.

Gene transfer technology, or gene therapy, in which foreign DNA is introduced into the host, resulting in the encoded protein being synthesized within the host's cells, addresses these issues: the encoded proteins will continue to be produced as long as the transgene persists and there is a much greater probability that they will be appropriately processed post translation and consequently fully functional. Nonetheless, a number of factors limit the efficacy of gene therapy approaches, such as low efficiency of gene transfer, cumbersome methodologies, and the inability to express the transgene at sufficiently high levels or to sustain its expression over time [101–

103]. Adenovirus has emerged as a favoured vector for gene transfer, as it exhibits near-complete transfection efficiency and high levels of transgene expression [104]. Unfortunately, the host immune system readily detects its viral origin and targets it for elimination [104, 105]. Antibodies against the common cold, which have been raised in the immune systems of most humans, also recognize the adenovirus vector [105]. These antibodies can inactivate the recombinant adenovirus at the outset of gene therapy, which not only limits transgene expression in the host, but may also elicit an acute inflammatory response in the wound [106], which could very well increase scarring.

Examples of either direct or vector-based administration of cytokines and other regulatory biomolecules that promote wound healing, which are regarded as being of particular interest, follow. Some other examples of vector-based administration [49, 107–111] are also noteworthy.

2.3.1 LL-37

The C-terminal fragment of human cathelicidin, the peptide LL-37, functions as a regulatory factor by inhibiting cytokine release to suppress the level of inflammation at the wound [112]. LL-37 has additional potent antimicrobial effects [113]. Wound infection can lead to enhanced inflammation and delayed wound closure, which produces more long-term scarring. Preventing or rapidly resolving wound infection not only attenuates inflammation, but also accelerates wound closure. LL-37 has also been shown to transactivate the epidermal growth factor receptor and thus stimulate keratinocyte proliferation. In an excision wound healing assay performed in diabetic mice, direct adenoviral transfer of LL-37 to cells at the perimeter of the wound significantly improved wound healing with reduced scarring compared to untreated controls [114].

2.3.2 Transforming growth factor-β3 (TGF-β3)

The cytokine “transforming growth factor beta” (TGF-β), which exists in three isoforms in mammals [115], controls proliferation, cellular differentiation, and other functions for most cells. The TGF-β signalling system has received much attention in the field of skin wound healing and appears to be a critical factor in the switch from the scar-free regenerative process in damaged embryonic tissues to the deposition of non-functional scar tissue that occurs in the post-natal healing process [9, 32–34, 115, 116]. Through considerable effort by many research groups, the molecular mechanisms by which TGF-β signal transduction pathways orchestrate the healing process have been dissected. TGF-β signalling is activated at the site of insult, where its roles include recruiting inflammatory cells, fibroblasts, and keratinocytes to the wound and stimulating neovascularization and vascular rearrangement [9, 32–34, 115, 116]. The relative abundance of TGF-β isoforms can shift the outcome from scar-forming to scar-free healing of skin wounds [116, 117]. Prolonged activation of the TGF-β1 and β2 pathways leads to scar formation [117, 118]. TGF-β1 is expressed predominantly in adult wounds; it is known to upregulate collagen synthesis and to reduce matrix metalloproteinase expression in fibroblasts [116], both of which result in scar tissue. In embryonic wounds, which heal without scarring, there is a predominance of TGF-β3 [119, 120]. These findings led to a pivotal study that demonstrated that treatment of wounds with exogeneous TGF-β3 inhibits scarring and promotes better collagen organization *in vivo* [116, 121]. In addition to its critical role in scar-less wound healing, recent reports demonstrate that TGF-β3 can also function as a cell homing molecule. In a study of articular

cartilage regeneration, application of TGF-β3-infused collagen gel recruited roughly 130% more endogenous stem cells to the wound site than collagen gel alone [122].

A recombinant TGF-β3 (avotermin or Juvista™) for use in scar revision surgery was under development until recently by the biopharmaceutical company Renovo Ltd. Human skin wounds treated with avotermin showed significant improvement in scar appearance both macroscopically and histologically, with improved restitution of the epidermis and an organization of dermal extracellular matrix more closely resembling normal skin [123]. However, despite its success in phase I and II clinical trials, avotermin failed to meet objectives in phase III trials, leading to the abandonment of its further development [124].

2.3.3 Interleukin (IL)-10

Interleukins (ILs) are a class of cytokines that are important in the recruitment and activation of inflammatory cells. For example, IL-6 is a chemotactic and stimulatory agent that attracts and activates monocytes and macrophages, while IL-8 attracts neutrophils and stimulates neovascularisation [125]. Although IL-6 and IL-8 levels are rapidly increased in the primary stages of both pre-natal and adult skin wounds, neither cytokine is detectable in fetal tissue 12 hours after wounding. In contrast, both cytokines remain highly expressed in adult wounds even 72 hours after wounding [126, 127]. This finding suggests that the prolonged expression and activity of IL-6 and IL-8 in adult wounds may contribute to scar formation, a conclusion which is supported by the finding that supplying exogenous IL-6 to fetal wounds is sufficient to induce scarring [126]. IL-10 is another cytokine that is expressed in the second and third phases of wound healing, where one of its key anti-inflammatory functions is to inhibit production of IL-6 and IL-8. Thus, IL-10 could potentially be used as an IL-6/IL-8 antagonist and anti-scarring agent. Direct administration of a recombinant adenovirus expressing IL-10 to adult mouse wounds reduced inflammation and produced scarless healing [128]. In addition, a recombinant human IL-10, trade name Prevascar, is currently in a phase II clinical trial [121].

2.3.4 Thymosin β4 (Tβ4)

Thymosins are small, naturally occurring polypeptides that act as biological response modulators (substances that regulate the activity of the immune system). They are so named because these were originally isolated from the thymus, but most are now known to be present in many other tissues. Tβ4, a 43 amino-acid peptide found in almost all types of cells, has many biological functions, which is unexpected for such a small peptide. Some of these functions are very important in wound healing. Tβ4 has antimicrobial properties, which help to prevent and fight wound infection [129]. In addition, Tβ4 decreases inflammation by down-regulating expression of inflammatory chemokines and cytokines [130, 131]. It also promotes cell migration and re-epithelialisation [132], reduces cell death [133] and promotes stem cell differentiation [134]. Lastly, Tβ4 stimulates neo-vascular formation [135]. Through these diverse and pivotal mechanisms, Tβ4 improves skin regeneration and reduces scar formation. In animal wound healing models, topical or systemic application of Tβ4 improved wound healing and reduced scarring. Unexpectedly, Tβ4 can also stimulate hair growth around the wound by activating existing hair follicles. This observation suggests that Tβ4 may stimulate epithelial stem cells residing in the hair follicle bulge, which are known to promote re-genesis in response to epithelial injury [136].

As a consequence of its ability to promote tissue and organ repair, the biopharmaceutical company RegeneRx has developed therapeutic applications of Tβ4 which are currently in clinical trials.

2.3.5 Stromal cell-derived factor-1α (SDF-1α)

The small cytokine SDF-1α is a member of the chemokine (C-X-C motif) ligand 12 (CXCL12) family. SDF-1α is the ligand for the cell surface receptor CXCR4 that is responsible for cell homing [137]. As such, SDF-1α is a critical factor for stimulating the mobilization and recruitment of stem cells that express the CXCR4 receptor on their cell surface [138, 139]. During normal embryonic development, SDF-1α and CXCR4 are expressed in complementary patterns in order to recruit stem cells to sites of rapid vascular expansion. SDF-1α has also been shown to be important in the recruitment of stem cells to human myocardium following ischemic injury [140]. Hypoxic gradients in the wound stimulate expression of the transcription factor hypoxia inducible factor-1α (HIF-1α) in a corresponding gradient, where HIF-1α levels are highest in the center of the wound and taper off at the perimeter. HIF-1α then induces expression of SDF-1α in a similar gradient [137]. CXCR4-positive stem cells are recruited to the wound by an increasing gradient of SDF-1α [137]. Treatment of impaired diabetic wounds, which do not establish a hypoxic gradient, showed improved healing when either HIF-α or SDF-1α was overexpressed in the wound [141, 142]. In a recent study, direct delivery of SDF-1α via hydrogel improved the rate and quality of wound healing in mice [143].

2.3.6 Homeobox A3 (HOXA3)

The transcription factor HOXA3 can significantly promote migration of endothelial cells and keratinocytes, which dramatically improves both angiogenesis and wound closure *in vivo* [144]. Local delivery of HOXA3 has been shown to selectively recruit stem and other progenitor cells to wounds and to inhibit expression of several members of the pro-inflammatory nuclear factor kβ pathway [145]. Thus, HOXA3 accelerates wound healing by recruiting stem cells and attenuating the excessive inflammatory response as well.

2.3.7 Decorin

Decorin is a small, naturally occurring, extracellular matrix proteoglycan, which is associated with collagen fibrils in all connective tissues. Decorin is required for the proper assembly of collagenous matrices [146–148]. Analysis of scar tissue from burn patients revealed delayed initiation of decorin expression [149] and an abnormally low amount of decorin compared with normal skin [149, 150]. The lack of decorin accounts for the poor organization of collagen fibrils typically associated with scar tissue. These effects can be attributed, at least in part, to its ability to modulate TGF-β signaling [147, 151–155]. Many studies have demonstrated the anti-fibrotic effects of decorin during wound healing in various contexts [154–159]. Recent reports have shown that direct injection of recombinant human decorin can efficiently prevent fibrosis and enhance tissue regeneration [156] and that decorin gene transfer promotes muscle regeneration [160]. Despite its success in animal models, attempts to develop a decorin-based therapy for clinical applications have been unsuccessful, due to the manufacturing challenges of producing

active decorin *in vitro* and failure to administer sufficiently large enough quantities for an appropriate duration to elicit an effect [147].

3 Discussion

In spite of recent advances in skin wound healing using MSC-based therapies, current approaches still fall short of producing scar-free tissue that has regenerated all of the structures and functions of normal skin. Although MSCs exhibit potent healing properties, two major capabilities require enhancement before they can achieve these physiological milestones. First, their anti-inflammatory properties require improvement, since prolonged, acute inflammation is the underlying cause of SIRS and of scar formation, and current MSC-based therapies do not eliminate scarring. Second, their capability to regenerate epithelial cell lineages, including skin appendages, needs to be enhanced before MSC applications can achieve optimal wound healing.

Administration of a number of cytokines and other regulatory biomolecules to skin wounds has been shown to positively influence wound healing. Unfortunately, the positive effects are of limited duration, since such proteins are generally subject to rapid degradation. In addition, direct administration of such regulatory biomolecules as a method of therapy and not as a research tool is not practical. Gene therapy, in which foreign DNA is introduced into the host, resulting in the encoded protein being synthesized within the host's cells, addresses these issues: the encoded proteins will continue to be produced as long as the transgene persists and there is a much greater probability that they will be appropriately processed post translation and consequently fully functional. A number of factors limit the efficacy of gene therapy approaches, however, such as low efficiency of gene transfer, cumbersome methodologies, and the inability to express the transgene at sufficiently high levels or to sustain its expression over time.

In addition, while adenovirus has emerged as a favoured vector for gene transfer, as it exhibits near-complete transfection efficiency and high levels of transgene expression, the host immune system readily detects its viral origin and targets it for elimination. Antibodies against the common cold can inactivate the recombinant adenovirus at the outset of gene therapy, which not only limits transgene expression in the host, but may also elicit an acute inflammatory response in the wound, which could very well increase scarring.

Human MSCs, however, are a promising delivery vehicle for recombinant adenovirus for two reasons: MSCs avoid immune-detection by the host, and have been shown to persist within the host for periods of several months [63, 161]. DRDC Suffield has successfully uploaded MSCs with a genetic payload that produces therapeutic levels of antibody *in vitro*, and are currently evaluating and optimizing the therapeutic effects of MSC-mediated gene transfer in a mouse model. It intends to develop a therapeutic approach for scar-less regeneration of massive burn wounds that couples the innate healing power of applied MSCs with the targeted delivery of factors known to minimize scar formation and promote skin regeneration. Gene transfer technology will be combined with MSC transplant therapy by first transducing cultured MSCs with transgenes that encode wound-healing factors, and then transplanting the genetically modified MSCs at the wound site. This is a novel approach in which MSCs can provide a dual function, to seed cells for tissue regeneration and to deliver genes that promote wound healing. Although there is a paucity of literature describing the use of genetically modified MSCs in the treatment of skin wounds, it is believed this is a feasible approach with significant potential to improve the prognosis for patients with severe skin wounds.

The initial focus will be to improve the capacity of MSCs to reduce scarring and to regenerate skin appendages like hair follicles, sweat glands and sebaceous glands. There are two classes of genes to be considered to achieve these physiological outcomes. Genes that encode factors with anti-scarring effects will typically have roles in counteracting inflammation [22, 110, 162]. Rapid resolution of the inflammatory phase accelerates wound closure and minimizes the natural over-production of matrix molecules used to contract the wound, which later gives rise to scar tissue. Genes that encode proteins capable of recruiting endogenous stem cells, especially epithelial progenitors, to the wound would improve the regenerative capacity of the injured skin [136, 162–164].

Of the regulatory proteins evaluated in this study

- LL-37 would be an ideal candidate for MSC-mediated gene transfer, as the predicted outcome of further reducing scar formation compared to MSC transplant alone;
- IL-10 may significantly improve healing, by combining the anti-inflammatory effects of IL-10, which rapidly resolve the acute wounding phase and minimize scarring, with the re-epithelialisation and neo-vascularisation effects of MSCs;
- SDF-1α is a potential candidate for MSC-mediated gene transfer, as its direct administration improves the rate and quality of wound in mice;
- Delivery of decorin to the wound by MSC-mediated gene therapy would circumvent the current problems in its clinical roadblock; and
- Although there was a failure of Renovo's TGF-β3 therapy to reduce scarring in a surgical setting, delivery of TGF-β3 by MSC-mediated gene therapy has the potential to produce scar-free healing and promote regeneration of injured tissue.

4 Conclusion

Current approaches to skin wound healing using therapies employing mesenchymal stem cells (MSCs) still fall short of producing scar-free tissue that has regenerated all of the structures and functions of normal skin. Genetic engineering of MSCs holds great promise for overcoming these hurdles. Uploading MSCs with a genetic payload to produce selected anti-inflammatory and regenerative signalling molecules directly in the wound would enhance their innate ability to quickly resolve the inflammatory phase, recruit and activate endogenous stem cells to repopulate the wound, and regenerate all the normal skin appendages and restore full function to the wounded casualty. The potential therapeutic applications generated from this project are expected to be vital tools which would alleviate the burden of Canada's over-extended burn specialists and improve the outcome for Canadian Armed Forces (CAF) casualties and civilians suffering from severe burn injury.

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List of symbols/abbreviations/acronyms/initialisms

CAF	Canadian Armed Forces
DND	Department of National Defence
DRDC	Defence Research and Development Canada
DRDKIM	Director Research and Development Knowledge and Information Management
ECM	Extracellular matrix
HIF-1α	Hypoxia inducible factor-1α
HOX3A	Homeobox 3A
IL	Interleukin
MSCs	Mesenchymal stem cells
R&D	Research and development
SDF-1α	Stromal cell-derived factor-1α
SIRS	Systemic inflammatory response syndrome
Tβ4	Thymosin β4
TGF	Transforming growth factor
TBSA	Total body surface area

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2. SECURITY CLASSIFICATION (Overall security classification of the document including special warning terms if applicable.)

UNCLASSIFIED
(NON-CONTROLLED GOODS)
DMC A
REVIEW: GCEC June 2010

3. TITLE (The complete document title as indicated on the title page. Its classification should be indicated by the appropriate abbreviation (S, C or U) in parentheses after the title.)

Developing Mesenchymal Stem Cell-Based Therapy for Severe Skin Burn Wounds

4. AUTHORS (last name, followed by initials – ranks, titles, etc. not to be used)

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5. DATE OF PUBLICATION (Month and year of publication of document.)	6a. NO. OF PAGES (Total containing information, including Annexes, Appendices, etc.)	6b. NO. OF REFS (Total cited in document.)
April 2012	46	164

7. DESCRIPTIVE NOTES (The category of the document, e.g. technical report, technical note or memorandum. If appropriate, enter the type of report, e.g. interim, progress, summary, annual or final. Give the inclusive dates when a specific reporting period is covered.)

Technical Memorandum

8. SPONSORING ACTIVITY (The name of the department project office or laboratory sponsoring the research and development – include address.)

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9a. PROJECT OR GRANT NO. (If appropriate, the applicable research and development project or grant number under which the document was written. Please specify whether project or grant.)	9b. CONTRACT NO. (If appropriate, the applicable number under which the document was written.)

10a. ORIGINATOR'S DOCUMENT NUMBER (The official document number by which the document is identified by the originating activity. This number must be unique to this document.)	10b. OTHER DOCUMENT NO(s). (Any other numbers which may be assigned this document either by the originator or by the sponsor.)
DRDC Suffield TM 2012-028	

11. DOCUMENT AVAILABILITY (Any limitations on further dissemination of the document, other than those imposed by security classification.)

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12. DOCUMENT ANNOUNCEMENT (Any limitation to the bibliographic announcement of this document. This will normally correspond to the Document Availability (11). However, where further distribution (beyond the audience specified in (11) is possible, a wider announcement audience may be selected.))

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One of the most common battlefield afflictions is severe skin injury, including burn trauma. Although medical strategies have been developed to improve burn wound healing, current approaches still fall short of restoring normal appearance and function in the case of third-degree (full-thickness) wounds. In the normal healing process, skin wounds do not regenerate the damaged tissue, but rather replace it with scar tissue which lacks normal function and appendages such as hair follicles and subcutaneous glands. The formation of fibrotic scars after wounding poses a major medical problem and expense. Skin scarring and appendage deficiency, following wound healing, often result in loss of skin function, restriction of mobility leading to disability, and adverse psychological effects, all of which severely affect the quality of life for patients. Application of mesenchymal stem cells (MSCs) to excision and burn wounds has been shown to improve the rate and integrity of the natural healing process. Mounting evidence suggests that transplanted MSCs recruit endogenous stem cells to the site of injury and attenuate the inflammatory response of the host through a paracrine (localized) response. Importantly, human MSCs are immune-privileged, and thus can be transplanted between individuals without rejection. However, current MSC-based therapies do not produce seamless skin regeneration, suggesting that the innate ability of MSCs to improve wound healing is limited. The ability of therapeutic MSCs to regenerate functional tissue might be improved through genetic modification, in which MSCs are engineered to deliver factors known to minimize scar formation and promote skin regeneration. In this manner, the innate healing power of applied MSCs can be coupled with gene therapy to fully realize the possibility of seamless skin wound repair and regeneration.

Les lésions cutanées graves, dont les brûlures, comptent parmi les affections les plus courantes sur le champ de bataille. Malgré la mise au point de stratégies médicales en vue d'améliorer la cicatrisation des brûlures, les méthodes actuelles ne permettent toujours pas de rétablir l'apparence et la fonction normales à la suite de lésions au troisième degré (profondes). Au cours du processus normal de cicatrisation, les tissus endommagés ne sont pas régénérés, mais plutôt remplacés par du tissu cicatriciel. Ce dernier est dépourvu de phanères, comme les follicules pileux et les glandes sous-cutanées, et il s'ensuit une perte fonctionnelle. De plus, la formation de cicatrices fibreuses après une blessure représente un problème médical important et coûteux. Une fois la lésion guérie, les cicatrices et la disparition des phanères entraînent souvent une perte de fonction de la peau, une restriction de la mobilité à l'origine d'incapacités et des effets psychologiques indésirables. Tous ces facteurs peuvent affecter grandement la qualité de vie des patients. L'application de cellules souches mésenchymateuses (CSM) sur des plaies par excision ou par brûlure a permis d'améliorer la vitesse et l'intégrité du processus naturel de cicatrisation. Des données de plus en plus nombreuses indiquent que les CSM transplantées recrutent des cellules souches endogènes dans la région lésée et atténuent la réaction inflammatoire de l'hôte par un effet paracrine (local). Fait important, grâce au privilège immun, la transplantation de CSM humaines entre deux individus n'entraîne pas de rejet. Cependant, les traitements actuels à base de CSM ne permettent pas une régénération cutanée homogène, ce qui donne à penser les CSM ont une capacité intrinsèque limitée d'améliorer la cicatrisation des plaies. La capacité des CSM thérapeutiques à régénérer un tissu fonctionnel pourrait être intensifiée par modification génétique, de sorte que les CSM libèrent des facteurs qui limitent la formation de cicatrices et qui favorisent la régénération cutanée. Ainsi, le pouvoir de guérison intrinsèque des CSM appliquées pourrait être combiné à la thérapie génique pour rendre possibles une réparation et une régénération optimales des plaies cutanées.

14. KEYWORDS, DESCRIPTORS or IDENTIFIERS

skin injury; burn injury; healing; stem cells; mesenchymal stem cells; gene therapy; genetic engineering