Cytotherapy, 2018; 0001–15

International Society for Cellular Therapy
ISCT®

Pericytes reduce inflammation and collagen deposition in acute wounds

RICHARD J. BODNAR[1,2], TIANBING YANG[3], LORA H. RIGATTI[4], FANG LIU[5], ALEXANDER EVDOKIOU[7], SANDEEP KATHJU[6] & LATHA SATISH[7,8,9]

[1]*Veterans Affairs Medical Center, Pittsburgh, Pennsylvania, USA,*[2]*Department of Pathology, University of Pittsburgh, Pittsburgh, Pennsylvania, USA,*[3]*Department of Ophthalmology, Pittsburgh, Pennsylvania, USA,*[4]*Division of Laboratory Animal Resources, University of Pittsburgh, Pittsburgh, Pennsylvania, USA,*[5]*Human Genetics, Graduate School of Public Health, University of Pittsburgh, Pittsburgh, Pennsylvania, USA,*[6]*McGowan Institute for Regenerative Medicine, Pittsburgh, Pennsylvania, USA,*[7]*Research Department, Shriners Hospitals for Children, Cincinnati, Ohio, USA,*[8]*Department of Pathology and Laboratory Medicine, University of Cincinnati, Cincinnati, Ohio, USA, and* [9]*Department of Plastic Surgery, University of Pittsburgh, Pittsburgh, Pennsylvania, USA*

Abstract

Background: Pericytes have been shown to have mesenchymal stromal cell–like properties and play a role in tissue regeneration. The goal of this study was to determine whether the addition of a pericyte sheet to a full-thickness dermal wound would enhance the healing of an acute wound. *Methods:* Human muscle-derived pericytes and human dermal fibroblasts were formed into cell sheets, then applied to full-thickness excisional wounds on the dorsum of nu/nu mice. Histology was performed to evaluate epidermal and dermal reformation, inflammation and fibrosis. In addition, real-time reverse transcriptase-polymerase chain reaction (RT-PCR) was used to determine cytokine response. *Results:* Pericytes were detected in the wounds until day 16 but not fibroblasts. Decrease in wound size was noted in pericyte sheet-treated wounds. Enhanced neo-vascularization and healthy granulation tissue formation were noted in the pericyte-treated wounds. Expression of type I collagen messenger RNA (mRNA) was significantly higher in the fibroblast-treated group, whereas Type III collagen mRNA showed significant increase in the pericyte group at days 3, 6 and 9 compared with the fibroblast and no-cell groups. Trichrome staining revealed thick unorganized collagen fibrils in the fibroblast-treated wounds, whereas pericyte-treated wounds contained thinner and more alligned collagen fibrils. Tumor necrosis factor (TNF)-α mRNA levels were increased in the fibroblast-treated wounds compared with pericyte-treated wounds. *Discussion:* The addition of pericytes may confer beneficial effects to wound healing resulting in reduced recruitment of inflammatory cells and collagen I deposition, potential to enhance wound closure and better collagen alignment promoting stronger tissue.

Key Words: *excisional wounds, fibroblasts, pericytes, transplantation, type I collagen, type III collagen, vascularization*

Introduction

Skin protects the body against environmental influences and prevents water loss. The loss of this barrier through burns, trauma and surgical excision requires the elementary processes of tissue repair for any organism to survive. Wound healing is a natural adaptive response to tissue injury. However, it remains a big challenge to enhance the healing of chronic ulcers and to prevent scarring in acute traumatic wounds [1]. In skin wounds of healthy adults, the barrier function of skin is efficiently restored; however, repair of deep dermal structures culminates in scar formation with loss of the original tissue structure and function. Wounds that exhibit impaired healing frequently enter a state of pathological inflammation due to a delayed, incomplete or uncoordinated healing process [2]. Although no two wounds are the same, there is a commonality in the dysfunction of specific factors promoting the formation of chronic wounds and scars. Studies of fetal skin wounds and oral mucosa that heal exceptionally quickly and with low/no scar formation show a reduction in inflammation and minimal fibrosis [3].

The ability to encourage cutaneous injuries to heal with limited to no scarring would significantly enhance healing of lacerations, incisions and burns, reducing morbidity and mortality rates. Current treatments only slightly improve tissue regeneration

Correspondence: **Latha Satish**, PhD, Shriners Hospitals for Children, Research Department, 3229 Burnet Avenue, Cincinnati, OH 45229, USA. E-mail: lsatish@shrinenet.org

(Received 5 December 2017; accepted 22 June 2018)

ISSN 1465-3249
https://doi.org/10.1016/j.jcyt.2018.06.011

and integrity, resulting in a need for the development of new therapies to enhance wound healing [4,5]. One promising approach to enhance tissue regeneration and reduce fibrosis is the use of pericyte-based therapies [6,7].

Pericytes (mural cells or Rouget cells) are cells that reside on the outer surface of the microvasculature. The cells tightly encircle capillaries, arterioles and venules, regulating microvascular physiology, blood flow and inflammatory cell trafficking [8,9]. In addition to their ability to promote vessel stabilization and maturation, detachment from the vessel promotes dedifferentiation into a stem-like progenitor cell [10]. Pericytes are thought to be of mesodermal origin and display differentiation potential. They have been found to exhibit multi-lineage potential, having the ability to differentiate into adipocytes, chondrocytes and myoblasts [11,12]. However, the heterogeneity of pericytes has led to questions regarding the origins of these cells from different organs. Pericytes purified from muscle, adipose, placenta and other organs have been shown to repair and regenerate damaged or defective tissues [13–16]. Thus, pericytes share similarities to mesenchymal stromal cells (MSC) and have been hypothesized to be the *in vivo* counterpart of MSCs [16–18]. A study by Blocki *et al.* showed that pericytes represented a subpopulation of MSCs and that pericyte behavior is not intrinsic to all MSCs [19]. Additionally, a study by Chen *et al.* (2013) showed that pericyte homing to perivascular sites may support the long-term survival of the pericytes and thus enhance overall outcome [13].

In the central nervous system (CNS), microvascular pericytes are thought to have macrophage-like properties and possess the ability to perform at least some immune function [20,21]. Pericytes are thought to play a role in regulating cell activation including T cells and neutrophils. Their interactions with macrophages and fibroblasts may foster vascular expansion and granulation tissue formation [22]. Also, pericytes have been shown to signal to keratinocytes to promote re-epithelialization [23]. In excessive fibrosis, it has been suggested that a population of pericytes may migrate into the perivascular space and develop into collagen-synthesizing fibroblasts [24].

Pericytes represent a potent cell population in the skin that can regulate the skin microenvironment, promoting wound healing, vascularization and cellular activation [6]. Therapeutic use of pericytes has shown the ability to enhance muscle regeneration and improve the repair of lung, kidney, cardiac and CNS tissues [25,26]. These studies demonstrate a novel use for pericytes in tissue repair. Pericyte interaction with inflammatory, dermal and epidermal cells makes them an enticing therapeutic candidate for various type of acute wounds and non-healing chronic wounds. The contributions of pericytes to skin tissue repair and regeneration are not well understood. To determine the direct influence of transplanted pericytes on the wound environment, we developed a cell sheet free of biomaterials that contains a homogenous cell type composed of either fibroblasts or pericytes with an extracellular matrix protein secreted from the cells. This exogenous scaffold-free cell system can be directly transplanted to the wound site without additional factors. Cell sheets derived from cell populations other than pericytes have been applied to a variety of tissues including the skin, cornea, myocardium, blood vessels and muscle but have not had significant or consistent positive outcomes [27]. This study provides new evidence that a pericyte cell sheet enhances the acute wound-healing process. Herein we show a one-time application of a pericyte sheet to full-thickness wounds significantly changes the wound bed by altering the inflammatory, cytokine and collagen profile, enhancing the wound-healing process and reducing fibrosis.

Materials and Methods

Preparation of cell sheets

Hs68 fibroblasts were cultured in Dulbecco's Modified Eagle's Medium (DMEM; Gibco, ThermoFisher Scientific Inc.) supplemented with 10% fetal bovine serum (FBS; Gibco; Thermo Fisher Scientific Inc.). Human muscle-derived pericytes [11] were a kind gift from Dr. Bruno Peaúlt (University of California, Los Angeles, Los Angeles, California, USA) and were grown in pericyte growth medium (ZenBio, Inc.). Pericytes used in all experiments were between passage five and seven. The protocol for cell sheet formation was as previously described [28]. In brief, cells were trypsinized and plated at a density of 2×10^6 cells/3.5 cm on NUNC UpCell temperature-responsive culture dishes (Thermo Fisher Scientific Inc.) and cultured at 37°C and 5% CO_2. The cells were incubated for 48 h, at which the monolayer was confluent (all cells were in contact). The confluent monolayer was washed once with phosphate-buffered saline (PBS), and incubated at 37°C with 5 μmol/L 1,1′-Dioctadecyl-3,3,3′,3′-Tetramethylindotricarbocyanine Iodide (infrared (IR) lipophilic tracer, $DiIC_{18}(7)$ (1,1′-Dioctadecyl-3,3,3′,3′-Tetramethylindotricarbocyanine Iodide) (DiR); Invitrogen, Thermo Fisher Scientific Inc.) suspended in Dimethyl sulfoxide (DMSO) for 20 min and then washed with PBS. The intact cell sheet was detached from the culture dish by incubation at 25°C for 30 min. After detachment from the 3.5-cm dish the circular cell sheet was 10–11 mm in diameter