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Paracrine action of mesenchymal stromal cells delivered by microspheres contributes to cutaneous wound healing and prevents scar formation in mice

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Abstract

Background aims. Accumulating evidence suggests that mesenchymal stromal cells (MSCs) participate in wound healing to favor tissue regeneration and inhibit fibrotic tissue formation. However, the evidence of MSCs to suppress cutaneous scar is extremely rare, and the mechanism remains unidentified. This study aimed to demonstrate whether MSCs—as the result of their paracrine actions on damaged tissues—would accelerate wound healing and prevent cutaneous fibrosis. *Methods.* For efficient delivery of MSCs to skin wounds, microspheres were used to maintain MSC potency. Whether MSCs can accelerate wound healing and alleviate cutaneous fibrosis through paracrine action was investigated with the use of a Transwell co-culture system *in vitro* and a murine model *in vivo*. *Results.* MSCs cultured on gelatin microspheres fully retained their cell surface marker expression profile, proliferation, differentiation and paracrine potential. Co-cultures of MSCs and fibroblasts indicated that the benefits of MSCs on suppressing fibroblast proliferation and its fibrotic behavior induced by inflammatory cytokines probably were caused by paracrine actions. Importantly, microspheres successfully delivered MSCs into wound margins and significantly accelerated wound healing and concomitantly reduced the fibrotic activities of cells within the wounds and excessive accumulation of extracellular matrix as well as the transforming growth factor-β1/transforming growth factor-β3 ratio. *Conclusions.* This study provides insight into what we believe to be a previously undescribed, multifaceted role of MSC-released protein in reducing cutaneous fibrotic formation. Paracrine action of MSCs delivered by microspheres may thus qualify as a promising strategy to enhance tissue repair and to prevent excessive fibrosis during cutaneous wound healing.

Key Words: *fibrosis, mesenchymal stromal cells, microspheres, paracrine, wound healing*

Introduction

The development of excessive fibrosis after deep dermal injury is the unavoidable consequence of adult mammals [1]. Fibrotic tissue does not contain the structures and the tensile strength that are native to the dermis and results in a weak point that is susceptible to re-injury. Besides leading to the function detriment of skin, psychological suffering always occurs. Both patients and physicians would welcome even small improvements in scar appearance. Hence, there has been considerable effort to the development of potential therapies to promote scar-less wound healing.

Although current treatments to attenuate cutaneous scar formation are limited, basic research and lessons from the clinic have distinctly advanced our understanding on underlying mechanisms of cutaneous scar formation. During the wound-healing process, the imbalance of pro-inflammatory mediators will direct the skin cells in generating excessive accumulation of collagen within the wounds, leading to deficient remodeling of the wound matrix that will result in the

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formation of a scar [2]. Additionally, α-smooth muscle actin (α-SMA) is important for fibrosis because it sustains the myofibroblasts that form granulation tissue [3]. Transforming growth factor (TGF)-β also plays a major role in the fibrosis; TGF-β1 and TGF-β 2 induce cuaneous scarring, whereas TGF-β3 appears to inhibit this effect [4].

Mesenchymal stromal cells (MSCs), which appear to be mediators of wound healing, have emerged as an attractive candidate for tissue repair. In particular, the participation and benefits of MSCs in wound healing were reported to be regulated by paracrine signaling molecules, including attenuating inflammation and reprogramming the resident immunity in the wounds [5,6]. Moreover, MSC transplantation in other organs can inhibit fibrotic tissue formation and restore depleted tissue function [6–9]. However, data supporting the role of MSC-secreted paracrine factors in the inhibition of the fibrotic behavior of cells within skin wounds is absent.

This study aimed to demonstrate whether MSCs can accelerate wound healing and alleviate or prevent cutaneous fibrosis through paracrine action. To explore the possible mechanism, we co-cultured inflammatory cytokine–stimulated fibroblasts with MSCs through the use of a Transwell system to elucidate the paracrine effect of MSCs on the fibrotic activates of fibroblasts. We then investigated this anti-fibrotic effect *in vivo* in a murine model with dorsal full-thickness skin wounds. In addition, MSC engraftment and survival within a hostile skin microenvironment may also adversely affect the outcome of MSC therapy. Our previous study suggested that bone marrow–derived MSCs (BMMSCs) could maintain the potential and be delivered safely and effectively to a wound and kept viable in the wound microenvironment through microsphere-based technology [10,11]. Therefore, to further minimize the side effects of transplantation and improve the therapeutic effects, microspheres were used to deliver MSCs in the present study. Our results indicate that MSCs retained the function through microsphere delivery and thereby promoted the wound-healing process and ameliorated cutaneous fibrosis.

Methods

All animal procedures were approved under the guidelines of the Institutional Animal Care and Use Committee of Chinese PLA General Hospital (Beijing, China).

Cell isolation and culture

Fibroblasts were isolated from dermal layers of the skin of C57Bl/6 mice; the specimens were minced into small pieces of less than 0.5 mm. The tissue fragments were then washed six times with medium and distributed into plates in Dulbecco's modified Eagle's medium supplemented with 100 μg/mL streptomycin (MP Biomedicals), 100 units/mL penicillin (MP Biomedicals) and 10% fetal bovine serum (Sigma Aldrich) in a humidified incubator at 5% CO_2 and 37°C temperature. The medium was replaced every 5 days. Fibroblasts from passages 2 through 4 were used in experiments.

Bone marrow–derived MSCs

BMMSCs were isolated from femurs and tibias of C57Bl/6 mice. Cells were regularly assessed for the required expression of cell surface markers and their differentiation capacity into adipogenic, chondrogenic and osteogenic lineages and were cultured as described previously [10]. BMMSCs from passages 2 through 4 were used in experiments.

Microsphere preparation

Gelatin microspheres were generated as previously described [12]. All reagents or chemicals were of analytical grade (Shanghai Chemical Co) and were used without any further treatment or purification. Briefly, 2 mL of 10 wt% gelatin aqueous solution was added drop-wise into 30 mL of paraffin oil while the mixture was mechanically stirred at 800 rpm; the solution was then rapidly cooled by immersing in ice-water. The formed microspheres were filtered, washed with acetone and dried at room temperature. As the carrier for cell culture, the resulting biocompatible microspheres without protein were screened with a diameter that ranged from 50 to 150 μm; microspheres were sterilized by use of ethylene oxide gas for 6 h and were then washed with phosphate-buffered saline (PBS) three times. BMMSCs (1×10^6) were inoculated into 1 mL of culture medium containing 20 mg of microspheres in siliconized 12-well cell culture plates. After a culture time of 72 h, MSC-microsphere complexes were formed and used in experiments.

Assessment of MSC potential

Cell surface marker expression was examined as follows. Fluorochrome-conjugated (fluorescein isothiocyanate, phycoerythrin or allophycocyanin) anti-human antibodies CD29, CD34, CD44, CD45, CD90 and CD105 were purchased from eBioscience for flow cytometry. MSCs were harvested either on microspheres or on tissue culture plates for 72 h, washed with PBS and incubated with antigen-specific antibodies for 30 min at room