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Original Article

Transplanted mesenchymal stem cells are effective for skin regeneration in acute cutaneous wounds of pigs

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ABSTRACT

Introduction: We investigated the effects of mesenchymal stem cells (MSCs) on cutaneous wound healing in pigs in order to develop new therapies to enhance wound healing in humans.
Methods: We cultured bone marrow cells from the femurs of male pigs, and the multipotency of these cells were then confirmed. The characteristics of the cultured cells were determined by flow cytometric analyses. The MSCs were injected intradermally into the skin of pigs as auto-transplantation, and linear full-thickness incisional wounds were made through the injected area immediately afterward.
Results: The MSCs were found to be positive for SWC3a, CD44, SLA class I, CD29, CD44H, and CD90. At 28 days post-surgery, wounds treated with MSCs had healed well, with only very fine scars visible macroscopically. Histologically, collagen architecture was thick and elastic fibers appeared in the wounds. Histomorphologic scale analysis demonstrated that the wounds treated with MSCs scored better than the controls. Significantly larger fibroblasts were observed in the wounds treated with MSCs than controls.
Conclusion: These results indicate that transplantation of MSCs causes wounds to heal almost completely, possible indicating regeneration to normal skin. We hypothesize that the transplantation protocol described in this study may also be applicable to human wound healing.

1. Introduction

Recent reports have shown that bone marrow-derived mesenchymal stem cells (MSCs) are multipotent cells, and can be induced in vitro and in vivo for the regeneration and repair of damaged tissue in almost all major organs of the body, including the heart, brain, lung, liver, kidney and eyes [1,2]. MSCs have also been investigated as a potential therapy in cutaneous wound healing. For stem cell transplantation therapy, MSCs and embryonic stem cells (ESCs) have been most common cell types used, and they have been effective in promoting wound healing when injected into wound sites, alone or in combination with biological materials [3–5]. However, the objectives of most of these studies were to increase acceleration of wound closure for chronic non-healing wounds, such as diabetic ulcers or burns.

The biological response to wounds in higher organisms falls into two categories: regeneration and wound repair [6]. We have studied regeneration in wounds with the aim of suppressing scar formation after surgery. It is known that the fetal skin of mice possesses a regenerative activity before embryonic day 13, and that dermal mesenchymal cells are vital in this process [7]. Accordingly, the local application of multipotent cells such as fetal mesenchymal

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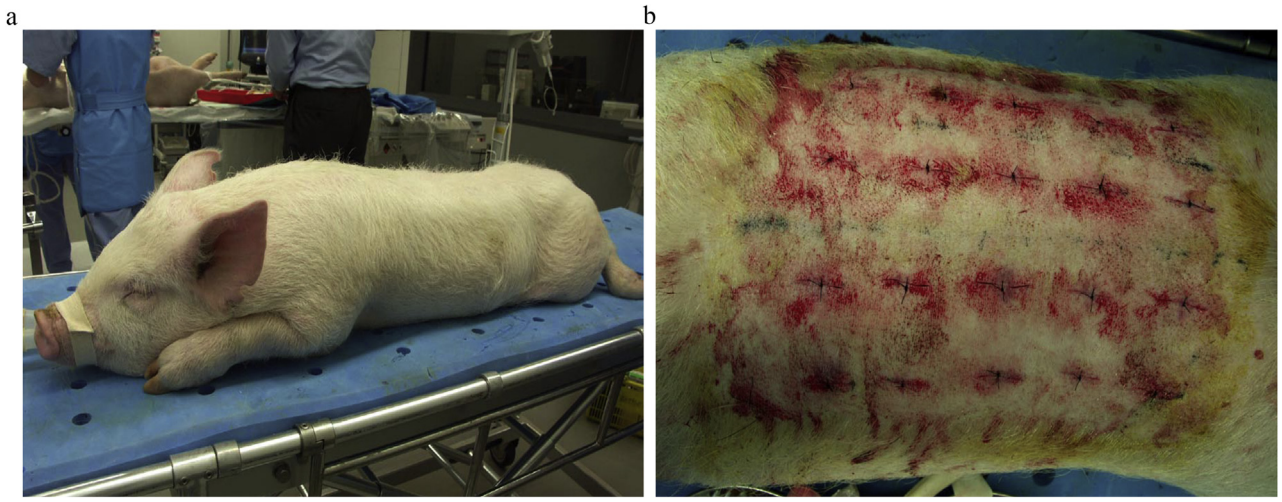

Fig. 1. Cell transplantation into incisional wounds of pigs. a. Bone marrow tissue was aspirated from the pelvises of two 4-month-old male L.W.D. pigs under general anesthesia. b. Cultured MSCs were suspended and injected intradermally into 20 points of the dorsal skin of each pig. Linear full-thickness incisions were created through the cell injected points. The wounds were closed with 4-0 nylon sutures.

cells on cutaneous wounds may result in wounds that heal without scars.

In a previous study, we transplanted rat MSCs into the incisional cutaneous wounds of rats [8] and found that wounds transplanted with rat MSCs had healed well, with very fine scars macroscopically. Significant differences were observed between the controls and the wounds transplanted with MSCs. These results indicate that wounds transplanted MSCs healed and resulted in skin that appeared to be essentially normal; thus, the process we induced was essentially regeneration of the structure of the skin.

In order to test the clinical application of our skin regeneration process, we investigated cutaneous wound healing in pigs. Pigs are often chosen to evaluate the biology of cutaneous wounds because they are an excellent model for human skin; they possess high similarity with humans in terms of anatomy, physiology, the wound healing response and chromosomal structural homology [9].

In the present study, we determined that acute cutaneous wounds in pigs treated with MSCs healed leave only very fine scars, and that MSCs can contribute to skin regeneration in such wounds. We hypothesize that further research may show similar effects in humans.

Score	2	1	0
Crust	Absent	Slightly present	Present
Texture	Present	Slightly present	Absent
Visible scar	Absent	Slightly present	Present

Fig. 2. Macroscopic assessment scale. Based on the images of digital camera and dermascope, scoring of wounds was performed according to the presence of crust, texture and visible scarring by three specialists in plastic surgery.

2. Materials and methods

2.1. Bone marrow cell preparation

This study was conducted according to the Guide for the Care and Use of Laboratory Animals published by the United States National Institutes of Health. The experimental protocols used for pigs were approved by the Animal Care and Use Committee of Showa University School of Medicine. Surgical treatments were

Epidermis	
0	normal
1	some restoration of rate ridges
2	no restoration of rate ridges

Dermis A. Collagen fiber orientation B. Collagen fiber density C. Collagen fiber maturity	
0	normal
1	<25% abnormal
2	26-50% abnormal
3	51-75% abnormal
4	76-100% abnormal
5	Keloid-like fiber

Fig. 3. Histologic assessment scale. Histologic assessment was done based on the method of Beausang et al. [12]. For evaluating epidermis, the presence of rate ridge was scored. For evaluating dermis, collagen fiber orientation, density, and maturity were determined.