DISCUSSION

Chronic wounds are difficult to heal, and little improvement has been shown in preventing the associated morbidity and disability in the past few decades [19]. The best available treatment for chronic wounds achieves only a 50% healing rate. It is true that the wound healing area in this study is also about 50% at control groups (Figure 3). Therefore innovative treatments to enhance wound healing and regeneration are needed. And the major goal of wound healing biology is to discover how skin can be induced to reconstruct damaged parts more perfectly [20]. Here we would show that SHED and hMSCs enhance wound healing by promoting re-epithelialization and the relationship of extracellular matrix, especially HA.

It was used an excisional wound splinting model, which prevented skin contraction and allowed wounds to heal through granulation and re-epithelialization [18], to study the effect of therapeutic reagents on wound healing. Our results indicate that wound closure in control groups is significantly delayed compared to hFibro, hMSCs, and SHED groups. And the implantation of hMSCs and SHED groups significantly promotes the wound healing process compared to hFibro and control after day 6 (Figures 2 and 3). Consistent with our findings, in previous reports it did not show enhancement of wound healing in allogeneic fibroblast-treated mice and showed that

MSCs promoted wound healing [21]. In short, the benefit of fibroblasts in wound healing remains controversial [22] and the fibroblasts were found to cause increased inflammation and scar formation [10]. On the other hand, MSCs were found to repair epithelium *in vitro* through differentiation and fusion [23] and the MSCs engrafted in the wound may contribute to cells in the skin epidermis and appendages, thus mediating dermal regeneration [21]. And previous study reported that a substantial engraftment of MSCs in the day 7 wounds and rapid reduction of the cells in the day 14 wounds. Our findings were consistent with these results, MSCs and SHED transplanted reduced from 7 to 14 days (Figures 4 and 5). The mechanisms involved with the decline of transplanted cells are not fully understood. With progression of wound healing process, cytokines and ECM molecules favorable to SHED and MSCs survival and engraftment would decrease [21]. And the cell which we injected by a turnover of the skin might come loose, and quantity of HA of ECM have decreased. A recent study showed HA peaked on highest levels at day 7 after post wound [24]. In this study it is also confirmed that HA expressed. The HA has important role in keratinocyte proliferation and migration, which implies important in the reepithelization process [25-27] and also reduce collagen deposition and therefore leading to reduced scarring [28].

Bone marrow aspiration for MSCs is an invasive and painful procedure for the donor.

In addition, it was wished effective treatments because it was confronted aging era at present. However the number, proliferation, and differentiation potential of MSCs decline with increasing age [12]. On the other hand, SHED contains a population of multipotent stem cell with the capacity to differentiate into several different cells lineages and expect that may substitute for MSCs [14]. But there is no prior reported about the influence on wound healing by local application of SHED. In this study, it could demonstrat that hMSCs and SHED accelerated wound healing compared to hFibro and control, too. As the result, SHED might become a useful cell origin as well as hMSCs.

In conclusion, our results showed the enhancement of wound healing with SHED significantly promotes wound healing compare to hFibro and control. This study demonstrated that deciduous teeth, which was considered as medical waste, would be novel therapeutic approaches in the treatment of wounds and novel stem-cell source for wound healing.

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Figure Legends

Figure 1. Schema of experimental protocol.

Figure 2. Effects of SHED on wound closure. Representative photographs of the wounds at day 7 and 14 after cell transplantation in murine excisional wound splinting model, in which wounds received implantation of PBS (control), hFibro, hMSCs or SHED. Left panel (a,c,e,g) is at day 7 after cell transplantation. Right panel (b,d,f,h) is at day 14 after cell transplantation. (a,b) control, (c,d) hFibro, (e,f) hMSCs, (g,h) SHED. Bars= 2mm

Figure 3. Wound measurement of control, hFibro, hMSC and SHED groups. Each point represents the mean of the percentage in area of the original wound size.

*Analysis of variance (ANOVA), versus cell transplantation groups or control *P<0.05*

Figure 4. Histologic evaluation for effect by transplanted cells at day 7. Left and middle panels showed hematoxylin and eosin staining, and right panel showed PKH 26-labeled cells (red fluorescence) and human HA staining (green fluorescence) and images were obtained fluorescence microscopy. DAPI was used to visualize the nuclei (blue

fluorescence). control (a,b,c), hFibro (d,e,f), hMSCs (g,h,i) and SHED (j,k,l). Original magnification (left panels ×40, middle panels ×400, right panels ×400, respectively). Bar=30mm (a, d, g, j); 25μm (b, c, e, f, h, i, k, l).

Figure 5. Histologic evaluation for effect by transplanted cells at day 14. Left and middle panels showed hematoxylin and eosin staining, and right panel showed PKH 26-labeled cells (red fluorescence) and human HA staining (green fluorescence) and images were obtained fluorescence microscopy. DAPI was used to visualize the nuclei (blue fluorescence). control (a,b,c), hFibro (d,e,f), hMSCs (g,h,i) and SHED (j,k,l). Original magnification (left panels ×40, middle panels ×400, right panels ×400, respectively). Bar=30mm (a, d, g, j); 25μm (b, c, e, f, h, i, k, l).

Table 1. Hyaluronic acid (HA) volume at day 7 and 14

	Day 7 (ng/mg)	Day 14 (ng/mg)
control	1558.4 ± 60.33	704.17 ± 40.26
hFibro	2092.75 ± 42.56	1425.79 ± 56.31
hMSC	2342.07 ± 188.10	1653.10 ± 120.84
SHED	2314.85 ± 164.91	1644.98 ± 120.70

*$p < 0.05$

Figure 1

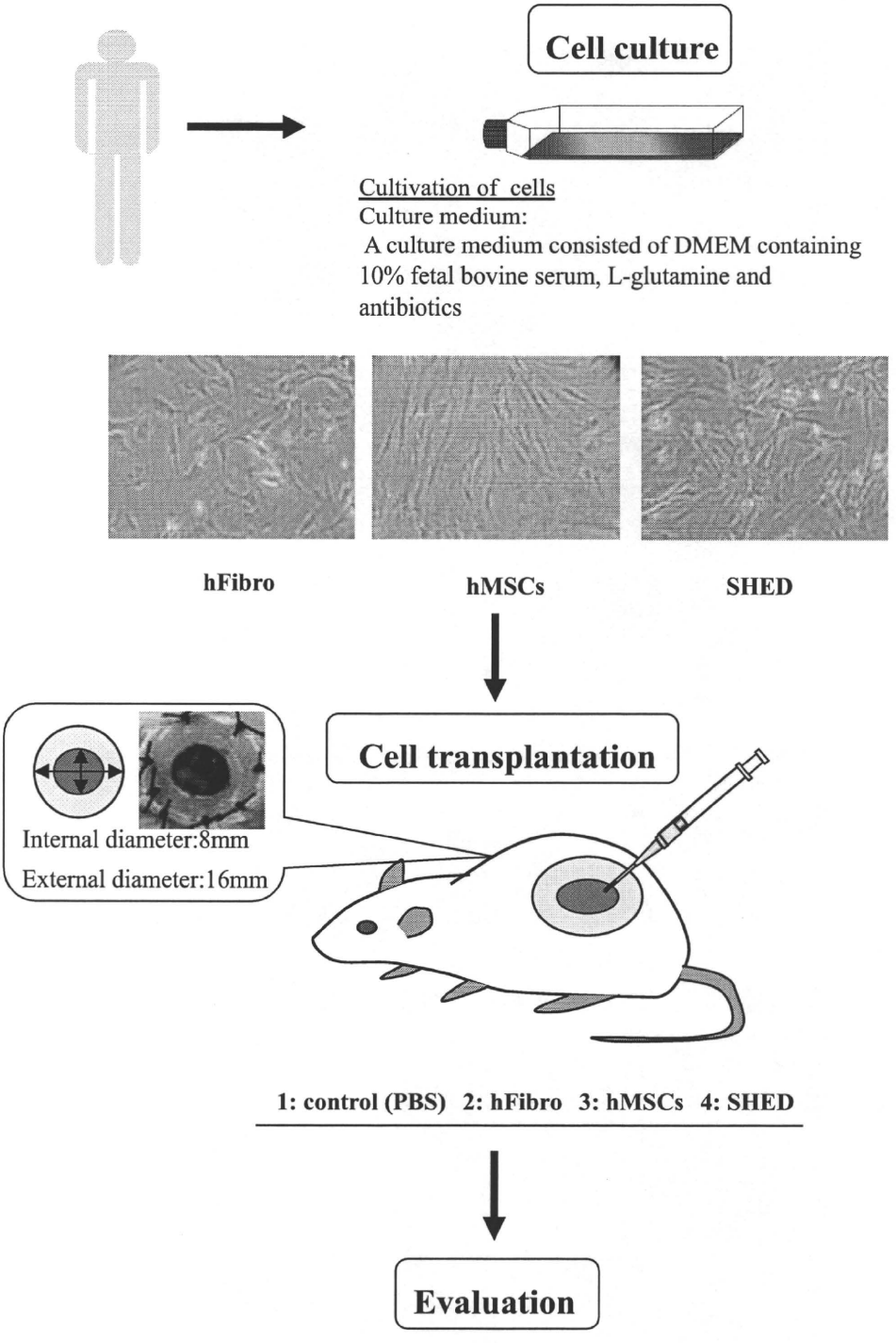

Cell culture
Cultivation of cells
Culture medium:
A culture medium consisted of DMEM containing 10% fetal bovine serum, L-glutamine and antibiotics
hFibro
hMSCs
SHED
Cell transplantation
Internal diameter:8mm
External diameter:16mm
1: control (PBS) 2: hFibro 3: hMSCs 4: SHED
Evaluation
1:Wound area analysis 2:Histology 3:ELISA

Figure 2

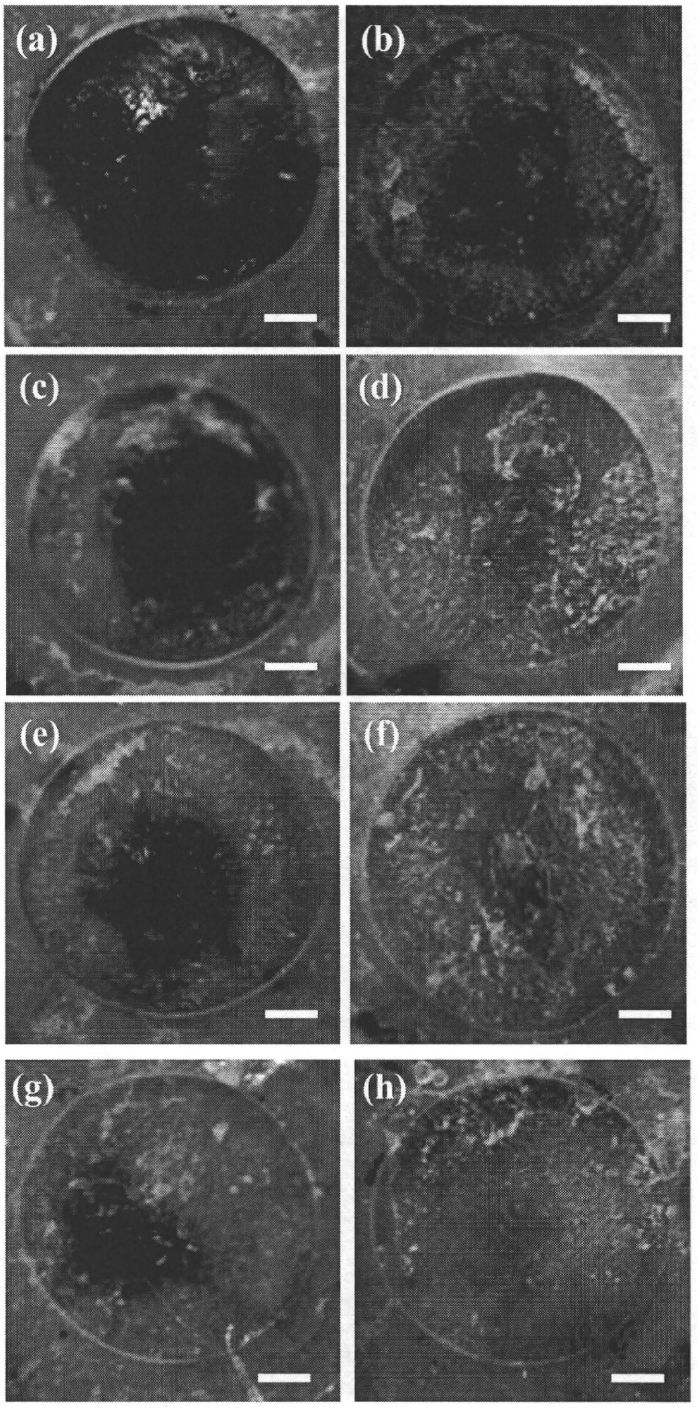


Figure 3

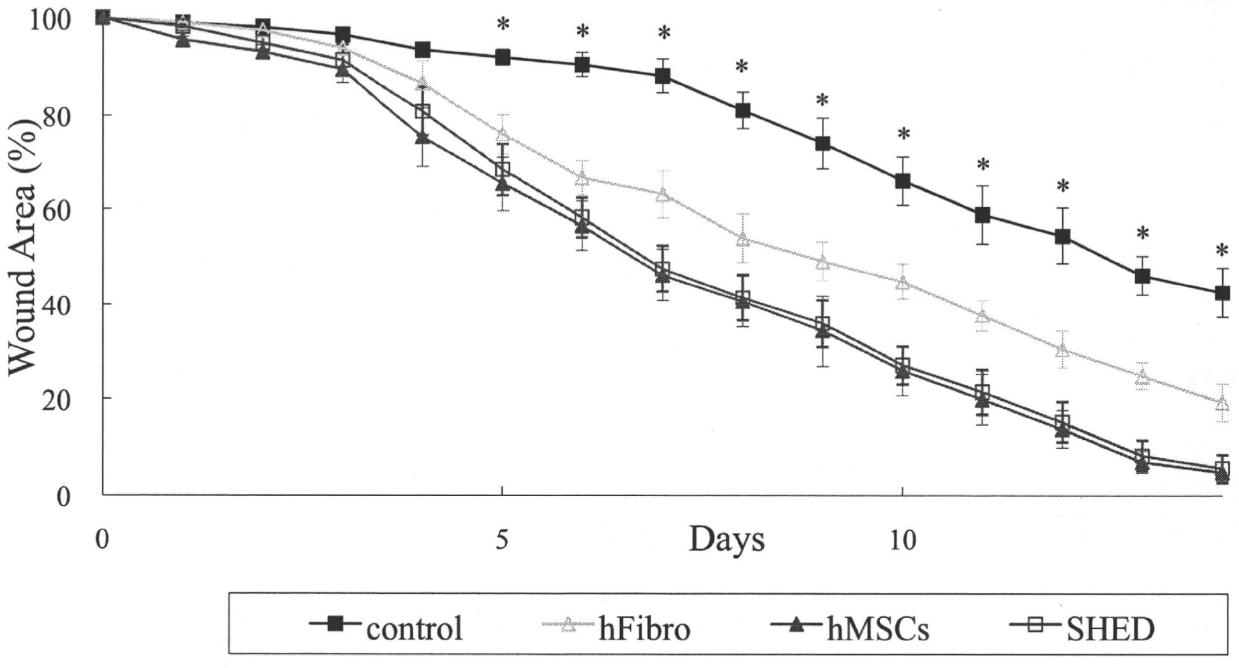

100
80
60
40
20
0
Wound Area (%)
0
5
10
Days
*
control
hFibro
hMSCs
SHED

Figure 4

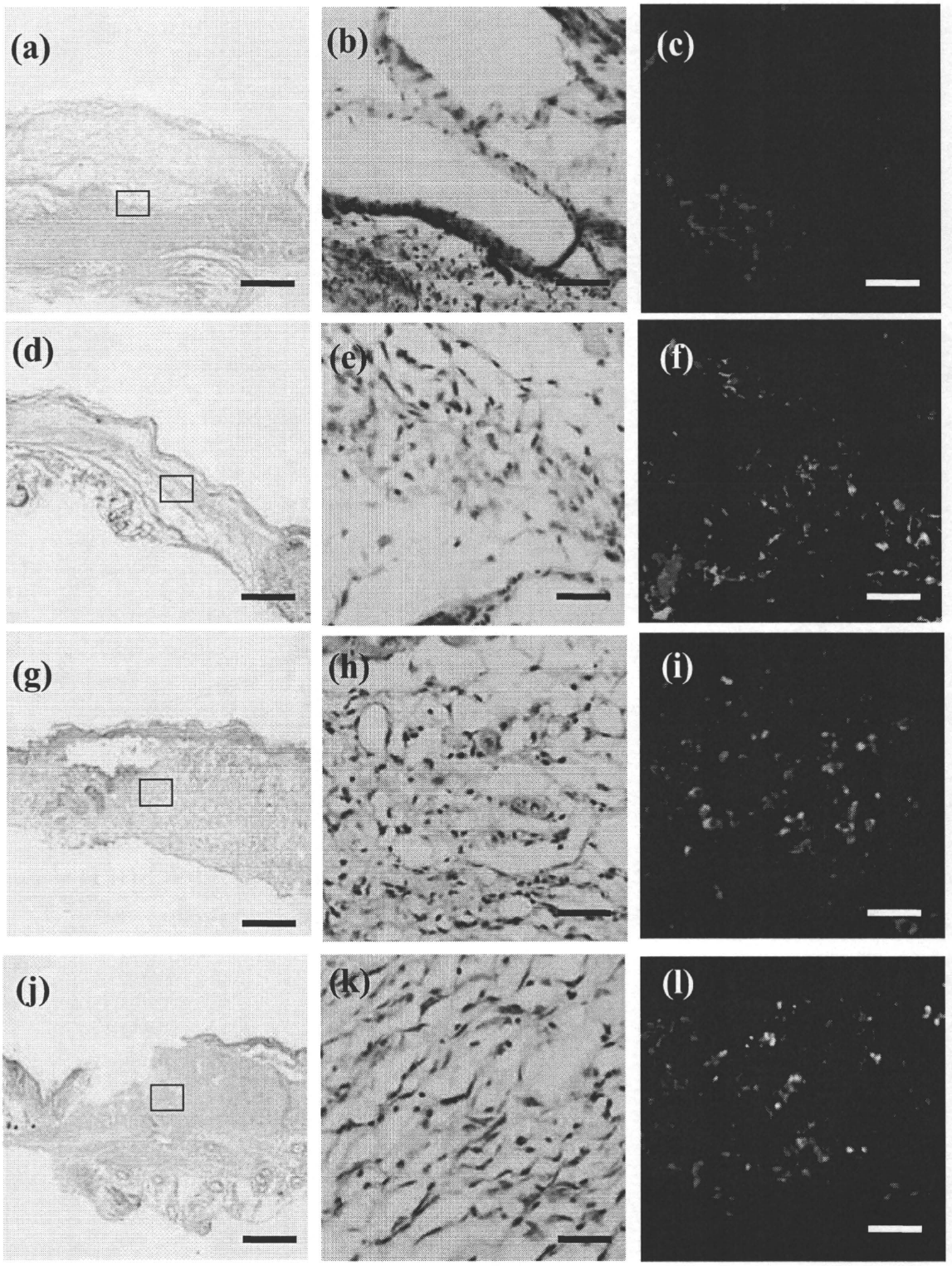
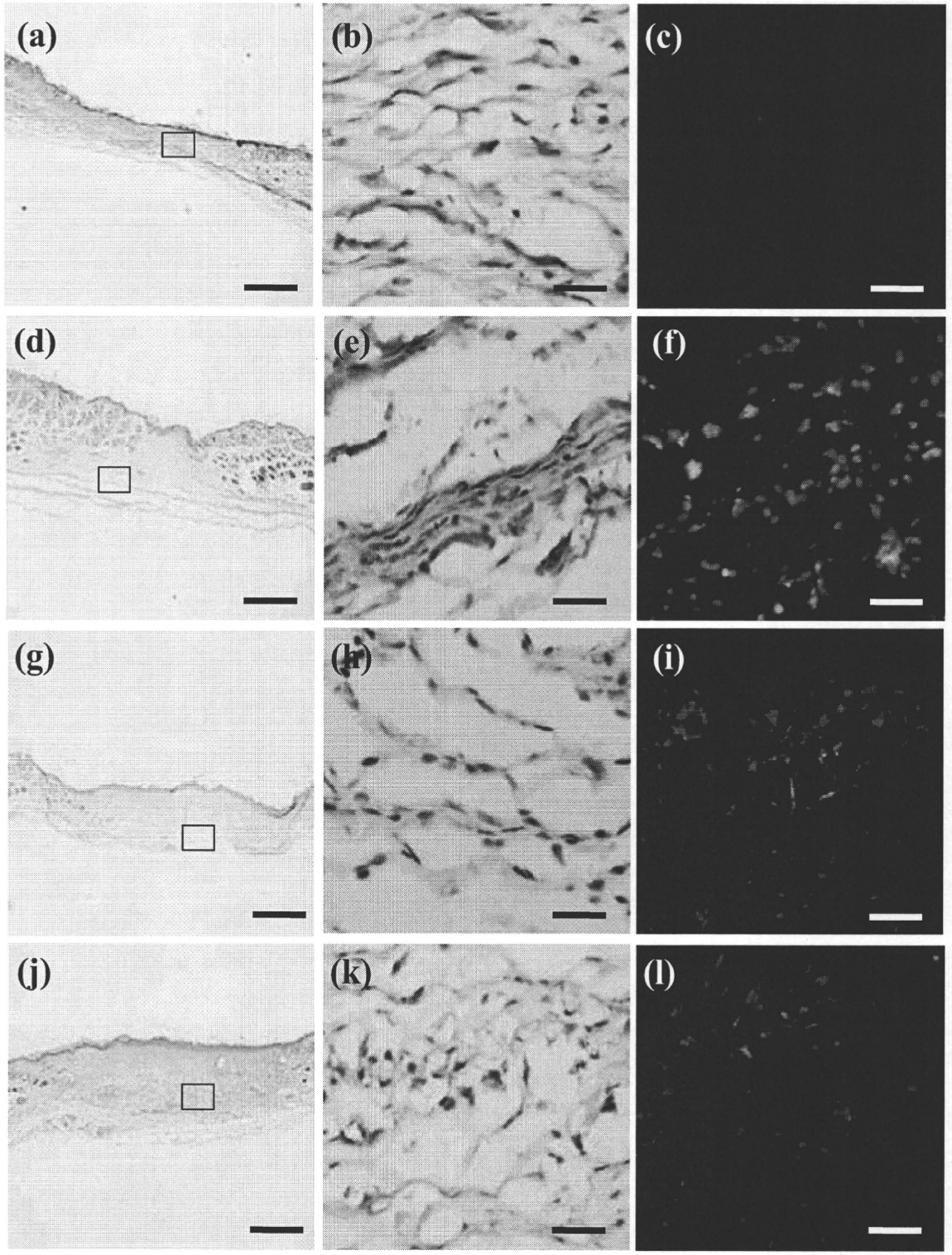


Figure 5



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*$p < 0.05$

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Cell-Based Cytokine Therapy for Skin Rejuvenation

Minoru Ueda, DDS, PhD, and Yudai Nishino, DDS

Abstract: The interaction between stem cells from human exfoliated deciduous teeth (SHEDs)–derived growth factors and human dermal fibroblast (HDF) has been investigated for the first time, as the application of SHEDs for dermal wound healing remains speculative. Stem cells from human exfoliated deciduous teeth have effects on HDFs by increasing collagen synthesis and by activating proliferation and migration activity of HDFs, suggesting that SHEDs or SHED-derived conditioned medium (SH-CM) can be used for the treatment of photoaging. Our results suggest that SHEDs and SH-CM should be constitutionally suited for photoaging treatment. Mainly with secreted growth factors or extracellular matrix proteins, SHEDs contribute to enhanced wound-healing potential of HDFs. Further mechanism studies using neutralizing antibodies against each growth factor may clarify the role of soluble factors of SHEDs in wound-healing process.

Key Words: Dental pulp stem cell, cytokine, skin rejuvenation, photo aging

(*J Craniofac Surg* 2010;21: 00–00)

Regenerative medicine using stem cells is a promising tool in a new clinical platform for a whole spectrum of intractable diseases. Various stem cells have been reported, including embryonic stem cells, induced pluripotent stem cells, and somatic stem cells. Because of ethical problems, immunorejection, and tumorigenesis, embryonic stem cells and induced pluripotent stem cells have many issues to overcome. Among them, somatic stem cells, especially mesenchymal stem cells (MSCs) isolated from various tissues including bone marrow (BMSCs), adipose tissue, skin, umbilical cord, and placenta,[1–4] have been used in clinical applications in skin regeneration. However, bone marrow aspiration is an invasive and painful procedure for the donor. In addition, the number, proliferation, and differentiation potential of BMSCs decline with increasing age.[5]

Dental pulp seems to be an alternative and more readily available source of stem cells. Stem cells from the pulp of permanent teeth (dental pulp stem cells [DPSCs]) and from exfoliated deciduous teeth (SHEDs) have been identified as a novel population of stem cells that have the capacity of self-renewal and multilineage differentiation and similar to BMSCs.[6,7] AQ1

Moreover, DPSCs have been reported to have the potential for use in cell-based therapy for systemic disease, such as neurologic disease and cardiac disease, and to ameliorate ischemic disease.[8–10] The main advantage of using SHEDs is that it can be obtained noninvasively from deciduous teeth that are routinely extracted in childhood and generally discarded as medical waste without any ethical concerns. Despite extensive reports on DPSCs, the characteristics and possible applications of SHED-derived stem cell remain poorly understood.

Recent studies have shown that MSCs may contribute to skin repair. In addition, there have been extensive investigations into wound healing by the exogenous application of various growth factors. However, result of utilizing growth factors at a single dose, multiple doses, or the combined application of multiple factors, with the expectation of synergistic effects, have not yet been confirmed clinically. Mixed growth factors secreted from stem cells may have an ideal combination that can improve damaged skin condition.

On the other hand, it has become the focus of cosmeceuticals and dermatologists treating an aging population overexposed to the sun; various noninvasive treatments and topical cosmeceuticals have been used to treat some symptoms of photoaged skin, including wrinkles.[11–13] Despite numerous claims of the reversal of wrinkles, solid scientific evidence regarding this issue is limited. Aging can be divided into 2 categories: intrinsic and extrinsic. Extrinsic aging refers to components mediated by environmental factors, which include smoking, chemical exposure, and primarily UV-B exposure.[11,14,15] Extrinsic aging is characterized by fine and coarse wrinkling, roughness, dryness, laxity, and pigmentary lesion. This type of aging causes a decrease in epidermal thickness and atypia of keratinocytes. In the dermis, UV-B exposure has been shown to stimulate collagenase production by human dermal fibroblasts (HDFs) and to upregulate collagenase gene expression. This induces degeneration of collagen and deposition of altered elastic tissue, which is prominent as wrinkles and yellow discoloration of skin.[16,17] Lasers and several cosmeceuticals include derivatives of retinol, vitamin C, and topical growth factors, all of which are well known for inducing collagen synthesis from HDFs and have been used for the treating skin texture and wrinkling.[13]

In the previous studies, MSCs have been known to produce various cytokines such as vascular endothelial growth factor, hepatocyte growth factor, insulinlike growth factor, platelet-derived growth factor, and transforming growth factor β. Recently, the production and secretion of cytokines have been reported as an essential function of MSCs, and diverse pharmacological actions of MSCs have been demonstrated especially in skin biology.[7,18–20] For example, it was reported[21] that MSCs have cutaneous healing effect through the production of diverse growth factors. These growth factors activated HDFs, which increase the proliferation/migration of HDFs and mediate the secretion of collagen from HDFs. The antioxidant effect of MSCs also has been demonstrated by showing that the secretory factors of MSCs protect HDFs from oxidative stress. The application of topical growth factors stimulated the repair of facial photoaging resulting in new collagen synthesis, epidermal thickening,

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