ASC-Derived Exosomes in Combination with Hyaluronic Acid Accelerate Wound Healing through Enhancing Re-epithelialization and Vascularization

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DEAR EDITOR,

Acute cutaneous wounds remain prevalent and demanding in dermatology. Although stem cell-based treatment has therapeutic benefits in wound repair, the clinical application is still limited.[1] Exosomes have effect on promoting tissue regeneration and could be an alternative of stem cells like ASC-derived exosomes (ASC-Exos).[2-6] The retention of exosome in targeted area needs to be enhanced, and hyaluronic acid (HA) may serve as exosome immobilizer and wound dressing.[7] In this pilot research, we examined the effect of ASC-derived exosomes (ASC-Exos) combined with HA on acute cutaneous wound healing of nude mice.

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Human ASCs (hASCs) were isolated from subcutaneous fat aspirated from a 25-year-old woman's abdomen with informed consent as approved by the Ethics Committee of Shanghai Ninth People's Hospital. The ASC-Exos were isolated from hASCs as described by Zhang et al.[8] Full-thickness skin defects were created on female nude mice (6-8weeks, 20-22g) (Sippr-BK laboratory animal Co. Ltd., Shanghai, China) and divided into four treatment groups (ASC-Exo+HA group, ASC-Exo group, HA group and Blank group). All the wounds were photographed on day 3, 6, 9, 12 and 15 post-operation, and the wound areas were measured using ImageJ software. The wound closure rate was then calculated by the formula $(A_0 - A_t)/A_0 \times 100\%$, where A_0 refers to the initial wound area, and A_t refers to the wound area on various observation days. Skin specimens collected on the endpoint (day 15) were tested by hematoxylin-eosin (HE) staining, Masson staining, proliferating cell nuclear antigen (PCNA) and CD31 expression. One-way ANOVA was employed to evaluate significant differences among groups, and t-test was used for comparing two groups. In nude mice models, the wound closure rate of the ASC-Exo+HA group, the ASC-Exo group, the HA group and the Blank group measured 97.73±1.54%; 92.37±3.84%; 88.83±2.66%; 80.07±2.91%, respectively. Consequently, the most prominent wound closure was observed in the ASC-Exo+HA group, while the ASC-Exo group exhibited less closure, and healing in the HA group and the Blank group was poor throughout the follow-up.

As demonstrated by the HE staining results, eminent skin renewal involving stratified epithelium, spindle-shaped fibroblasts and well-arrayed collagen fibers was observed in the ASC-Exo+HA group. The ASC-Exo group displayed granulation but lacked epidermal thickening and collagen ordering. Distinct inflammation was found in both the HA group and the Blank group (Fig. 1a, upper panel). The results of Masson staining revealed that collagen fibers were well arranged parallel to the skin surface in the ASC-Exo+HA group and the ASC-Exo group. Collagen fibers in the HA group were sparse and perplexed with swirling structures, and failure of collagen formation was observed in the Blank group (Fig. 1a, bottom panel).

The results of anti-PCNA staining showed that tissues from the ASC-Exo+HA group had greater numbers of PCNA-positive cells than the other groups in both epidermis and dermis, indicating that ASC-Exo+HA most significantly enhanced epidermal and dermal regeneration (Fig. 1b).

The results of anti-CD31 staining showed that in per spot view, the ASC-Exo+HA group demonstrated 18.0 ± 5.9 newly formed vessels; 11.4 ± 2.8 and 4.4 ± 2.3 vessels were discovered in the ASC-Exo group and the HA group, respectively, while 2.7±1.6 vessels were found in the Blank group (Fig. 1c, middle and bottom panel). Accordingly, the gross view of the dermal side of harvested skin

tissue exhibited the most prominent vascularization in the ASC-Exo+HA group, moderate in the Exo group and little in the HA and the Blank groups (Fig. 1c, upper panel).

This study proposed ASC-Exos as alternative for stem cells due to preparation convenience and absence of safety concerns. We further demonstrated that ASC-Exo+HA, as the form of exosomes combined with appropriate scaffolds, was effective and could be a promising clinical application for cutaneous wound. The results showed that ASC-Exo+HA could markedly promote fibroblast activities, re-epithelialization, vascularization in wound healing. However, there are still limitations. The mechanism how ASC-Exos promote proliferation and migration during wound repair course and in vivo tracing of ASC-Exos are still in need to be explored in future studies.

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