Effectively Inducing and Monitoring Adipose-derived Stem Cells-mediated Tissue Regeneration using a PEGylated Fibrin and Gold Nanoparticles

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Statement of Purpose: Stem cell treatment to skin wounds has shown to improve angiogenesis, extracellular matrix formation, and epidermal regeneration [1]. Adipose-derived stem cells (ASCs) are an attractive stem cell source due to their superior proliferation and angiogenic potential, relatively easy isolation, and sufficient quantity [2]. Using a natural polymer (*e.g.* collagen and fibrin) can activate cellular activities as well as stabilize localization of delivered stem cells in the wound [3]. An advanced hydrogel platform based on PEGylated fibrin (PFG) can be a promising tunable stem cell carrier with enhanced angiogenic potential and mechanical properties [4]. However, it is difficult to trace stem cells and noninvasively monitor the wound healing process of stem cells. Based on our previous studies, gold nanospheres were evaluated as an effective cell tracer for ASCs without any harmful effect on their activities such as proliferation and protein expression. The aims of the present study are to demonstrate 1) whether an ASCs-gold nanotracers (GNTs) seeded in a 3D PFG system in rat burn-injured wound can be traced/monitored using a dual biomedical imaging technique combining ultrasound and photoacoustic imaging modalities and 2) the healing effects (vascular or dermal differentiation and paracrine effects) of ASCs-GNTs on skin regeneration and angiogenesis in a burn wound.

Methods: Spherical burn wounds were created on the dorsal area of rats (Lewis, male, 7-11 week old) using a brass soldering device (~24 mm diameter and 500 g) that was set at 87°C for 10 seconds. For ASCs, adipose tissues were harvested from the fat pads of Lewis rats (male, 8-10 weeks, Harlan) and isolated by the enzymatic method using 0.05% collagenase type I. GNTs (20 nm) were acquired as described in Ricles *et al* [5]. In brief, 1 ml 10 mg/ml chroloauric acid (HAuCl4) (sigma) was added into 97 ml MilliQ water and boiled on a hot plate set at 400°C. Then, 2 ml 11.4 mg/ml sodium citrate (sigma) was added with rapid stirring. Cells were incubated with GNT-media solution at a concentration of 4×10^7/cell for 24 hours. For 2 ml PFG, 250 µl fibrinogen (80 mg/ml) solution in PBS (pH 7.8) was combined with 250 µl SG-PEG-SG solution (8 mg/ml) and 500 ul cell suspension (2×10^5 cells/ml, passage 3-6, CM-DiI fluorescent dye labeling) followed by addition with thrombin solution (25 U) diluted with calcium chloride at the volume ratio of 1:3. ASC-GNT-PFGs were implanted on the burn wound after eschar removal and the wound were covered using an occlusive dressing. Sham-treated controls were conducted identically by treating saline solution. Tissue samples were harvested on days 1, 4, 7, and 14 for analysis: wound closure measurement, histology (H&E stain and Masson's trichrome), western blots, immunostaining, photoacoustic/ultrasound imaging.

Results: Implanted gels were identified in the wound bed until day 7 but there were no remaining gel implants on day 14. Wounds were closed faster in sham-treated controls in day 7 than ASC-GNT-PFG groups but were similar on day 14 (data not shown). CM-DiI labeled ASCs were still detected in the gels and in the wound bed after one week (Fig. 1C). This localization of implanted stem cells was also monitored and visualized using ultrasound/photoacoustic imaging on the harvested wound skin (Fig.1B and D). Moreover, 3D spectroscopic images were selectively visualized skin, labeled cells and oxygenated/deoxygenated hemolgobin in the wound (Fig.1D). Histological analysis demonstrated the great coverage and integration with the wound, and the regeneration of more organized skin layer following ASC-GNT-PFG implantation compared to sham-treated (Fig.1E and F).

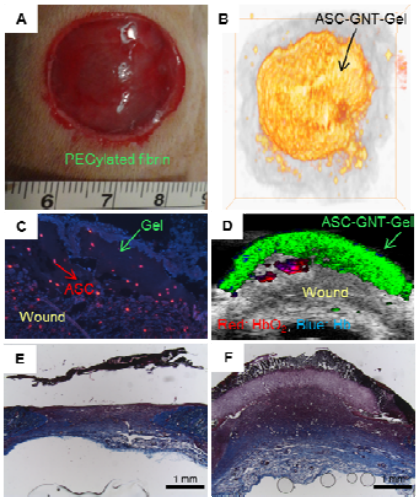

Figure 1. *In vivo* studies of ASC-GNT-PFG (A) Implantation of an ASC/GNT-implanted PFG in the rat burn wound (day 0). (B and D) Combined ultrasound and photoacoustic images of gels (day 7). (C) CM-diI-traced ASCs in the gel near the wound (day 7). (E and F) Masson's trichrome staining of sham-treated and ASC-GNT-gel groups (day 7).

Conclusions: Gold nanoparticles successfully traced ASC implants on burn wounds, suggesting this nanomaterial can be an effective and safe monitoring tool for noninvasive 3D imaging of stem cell-tissues. In addition, PEGylated fibrin with ASCs showed good dressing properties and improved tissue reconstruction. In the future, we will focus on detailed wound healing effect by immunostaining and western blots of paracrine, epidermal and vascular cell markers. In addition, we investigate the long-term wound healing effect (21 days).

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