Bilateral regulation of human monocytes and matrix-encapsulated mesenchymal stromal/stem cells in vitro and in full-thickness cutaneous wounds

David Antonio Cantu[1,2], Kedi Xu[1,7], Yao Fu[1], Jaehyup Kim[4], Xiaoxiang Zheng[7], Peiman Hematti[5], Weiyuan John Kao[1,2,6]
[1]School of Pharmacy, [2]Department of Biomedical Engineering, [4]Department of Cellular and Molecular Biology, [5]Department of Medicine, [6]Department of Surgery, University of Wisconsin-Madison, Madison, WI 53705, USA
[7]Department of Biomedical Engineering, Zhejiang University, Hangzhou 310027, PR China

Statement of Purpose: Allogeneic mesenchymal stromal/stem cells (MSCs) are being used in clinical trials for treatment of acute injuries and chronic illnesses however systemic administration in several studies failed to reach therapeutic endpoints[1]. Although prior investigations suggest that administered MSCs migrate towards sites of injury to mediate healing, several authors refute such claims showing poor spatial delivery and low engraftment efficiency[2]. Encapsulating biomatrices can enhance MSC retention and maintain their function to promote healing via extended secretion of growth factors and immunomodulatory cytokines[3]. *We hypothesize that the biomatrix can support MSCs' multidifferentiation potential and immunomodulatory properties on monocyte/macrophages to favorably promote cutaneous healing for a full-thickness wound defect.*

Methods: Free thiols were conjugated to gelatin (type B, bloom 225) by reacting gelatin lysyl residues to N-hydroxysuccinimide (NHS)-functionalized poly(ethylene)glycol (PEG-Bis-NHS), and cysteamine or L-cysteine to synthesize gelatin-PEG-SH (gel-PEG-SH). Gelatin/poly(ethylene)glycol biomatrices were formed by reacting gel-PEG-SH (10% wt/vol) with PEG diacrylate (PEGda, 10% wt/vol) and 2959 Irgacure photoiniator (0.5% wt/vol) for UV polymerization (λ_{max} = 365 nm, 100 W/cm^2, 2 min) for the *in vitro* studies[4]. Similarly, gel-PEG-SH (10% wt/vol) and PEGda (1% wt/vol) were polymerized via Michael-type addition (pH 8.5) for the *in vivo* investigation. Human bone marrow-derived MSCs were encapsulated in UV polymerized biomatrices while rat bone marrow-derived MSCs were incorporated into the Michael-type biomatrices (1x10^6 cells/mL). Multidifferentiation potential of human MSC was assessed (Adipocyte/Oil Red O, Chondrocyte/Safranin O, Osteoblast/Alzirin Red S staining) with cells still entrapped within the gelatin/poly(ethylene)glycol biomatrices using appropriate differentiation medias (14 day detection) in the presence or absence of human blood-derived monocyte/macrophages[4]. Protein expression of pro-/anti-inflammatory markers TNF-α, IL-6, -10, -12 were quantified to determine whether the MSC-biomatrix induces adherent monocyte/macrophages to adopt a pro-healing phenotype[5]. MSCs encapsulated within the gelatin/poly(ethylene)glycol biomatrix were applied as a dressing to full-thickness wounds in Sprague-Dawley rats, which were subsequently euthanized at 4 or 7 days. Wound resolution was assessed by determining the wound closure percentage, re-epithelialization thickness, and direct counts of different H&E stained cell types. Results were compared between the sham wound, gelatin/poly(ethylene)glycol hydrogel (Gel-PEG), and MSC-gelatin/poly(ethylene)glycol biomatrix (Gel-PEG-MSC) treatment groups.

Results: Biomatrix-encapsulated MSCs remained viable and demonstrated multidifferentiation potential into selected lineages and immunomodulation (TNF-α^{low}, IL-12^{low}, IL-10^{high}, IL-6^{high}) of monocyte/macrophages *in vitro*. The Gel-PEG-MSC treatment showed statistically greater wound closure and both the Gel-PEG and Gel-PEG-MSC treatments showed statistically greater epidermal thickness at 7 days. At 4 days, the Gel-PEG-MSC treatment showed attenuated polymorphonuclear leukocyte, mononuclear cell, and fibroblast infiltration. By 7 days, greater keratinocytes and fibroblasts densities were observed for the Gel-PEG and Gel-PEG-MSC treatments compared to the sham control. Poor biocompatibility indicated by the presence of foreign body giant cells or cell necrosis was not observed for either the Gel-PEG and Gel-PEG-MSC treatments.

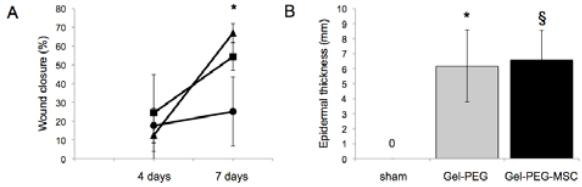

Figure 1. Wound closure percentage and epidermal thickness at 7 days.
A: The wound closure percentage was assessed for three different treatment groups (sham wound ●, Gel-PEG ■, and Gel-PEG-MSC ▲) 4 or 7 days post-surgery. *Gel-PEG-MSC demonstrated statistically greater wound closure than the sham control at 7 days.
B: 7 day full-thickness wounds for sham, Gel-PEG, Gel-PEG-MSC. *Gel-PEG and §Gel-PEG-MSC showed statistically greater epidermal thickness than the sham wound at 7 days. A probability $p < 0.05$ was considered statistically significant using student's t-test amongst treatment group means ± SD (n = 3).

Conclusions: The gelatin/polyethylene glycol biomatrix retained the MSCs and did not disrupt MSCs' multidifferentiation potential or immunomodulatory activity on biomatrix-adherent monocyte/macrophages *in vitro*. Retention of MSC therapeutic properties when encapsulated with the biomatrix enhanced cutaneous wound repair as observed by accelerated re-epithelialization and did not elicit an adverse foreign body response. Additional *in vivo* characterization will be undertaken to determine if the MSC-biomatrix biases macrophages towards a pro-healing phenotype favorable for wound resolution.

References:

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