Minocycline enhances the mesenchymal stromal/stem cell pro-healing phenotype in triple antimicrobial-loaded hydrogels for inoculated cutaneous wound healing

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Statement of Purpose: Mesenchymal stromal/stem cells (MSCs) have demonstrated pro-healing properties including an anti-inflammatory cytokine profile and the promotion of angiogenesis via expression of growth factors in pre-clinical models. MSCs encapsulated in poly(ethylene glycol) diacrylate (PEGdA) and thiolated gelatin poly(ethylene glycol) (Gel-PEG-Cys) crosslinked hydrogels have led to controlled cellular presentation at wound sites with favorable healing outcomes. However, the therapeutic potential of MSC-loaded hydrogels may be limited by non-specific protein adsorption on the delivery matrix that could facilitate the initial adhesion of microorganisms and subsequent virulent biofilm formation. Antimicrobials loaded concurrently in the hydrogels with MSCs could reduce microbial bioburden and promote healing, but the antimicrobial effect on the MSC wound healing capacity and the *in vivo* efficacy of the hydrogels in bacteria-inoculated wounds is unknown. In this study, we investigate the effects of minocycline, vancomycin, and linezolid on MSC wound healing characteristics and the antibacterial and wound healing efficacy in inoculated full thickness cutaneous wounds in a rat wound model. This combinational approach to biomaterial development has the potential to impact infection prophylaxis and advanced wound healing for full thickness cutaneous wounds.

Methods: MSCs were isolated from discarded filters of bone marrow harvests of healthy adult human donors based on a protocol approved by the University of Wisconsin Hospital and Clinics Regulatory Committee and characterized via flow cytometry and differentiation potential. Antibiotic doses for hydrogel formulations were based off of LIVE/DEAD® (Invitrogen) and CellTiter-Blue® (Promega) analysis. MSC invasion potential was determined through a CytoSelect™ Wound Healing Assay (Cell Biolabs, Inc.). MSC proliferation was determined through a CellTrace™ CFSE Cell Proliferation Kit (ThermoFisher Scientific) analyzed through flow cytometry. MSC gene expression was determined through RNA extraction using an RNeasy Mini Kit (Quagen) followed by reverse transcription using an iScript™ cDNA sysntehsis kit (Bio-Rad) and rt-qPCR using a StepOnePlus Real-Time PCR System (ThermoFisher Scientific). MSC adhesion to ECM proteins was determined using an ECM Array Cell Adhesion Assay (Cell Biolabs). MSC adhesion molecule and growth factor release was determined using respective ELISA kits (R&D Systems, Inc.) and angiogenesis induction potential was determined using human umbilical vein endothelial cells (HUVECs, ThermoFisher Scientific). Full thickness wounds were made on Sprague-Dawley rats using an 8-mm biopsy punch (Miltex GmbH) and inoculated with *Staphylococcus aureus* (SA) (ATCC strain #2913, OD_{600}=0.1). Hydrogels were made at 0.5% (w/v) Iragcure 2959 photoinitiator (BASF), 10% (w/v) PEGdA, and 10% (w/v) Gel-PEG-Cys with or without antibiotics and with or without MSCs at 100,000 cells/hydrogels. Bacterial growth, wound closure, and epithelial thickness of the wounds were determined at 1 and 3 days after treatment.

Results: MSCs were metabolically viable and maintained differentiation potential in hydrogels loaded at 50 µg/mL minocycline+40 µg/mL vancomycin+10 µg/mL linezolid. Minocycline at this dose alone and in combination with the other two antibiotics induced significantly higher gap closure after 24 hours of culture, expression of *LGALS1* and *TEK* after 48 hours in hydrogels, secretion of vascular endothelial growth factor (VEGF) after 48 hours in hydrogels, and attachment to fibronectin, collagen I, collagen IV, laminin I, and fibrinogen after 90 minutes of culture. HUVECs cultured with media pre-conditioned with MSC-loaded hydrogels loaded with minocycline alone or in combination with the other two antibiotics for 48 hours led to significantly higher HUVEC tube formation. All three antibiotics loaded in the hydrogels had no effect on MSC secretion of anti-inflammatory cytokines in response to inflammatory factors. Hydrogels loaded with MSCs and all three antibiotics demonstrated a significant decrease in SA Log CFU/mL and a significant increase in wound closure and re-epithelialization in SA-inoculated rat full thickness wounds.

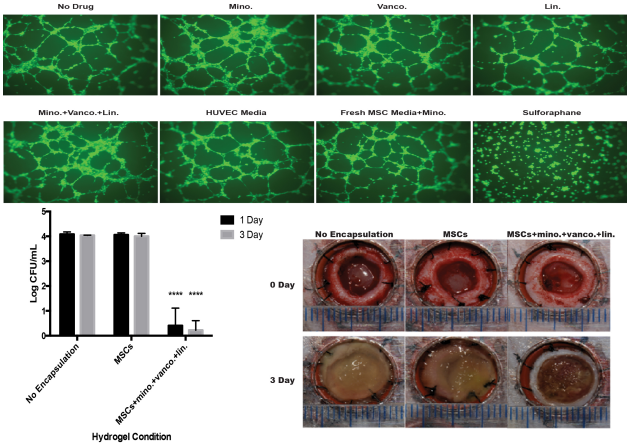

Figure 1. Angiogenesis induction potential and *in vivo* antibacterial and wound healing efficacy of MSC and antibiotic-loaded hydrogels

Conclusions: Minocycline induces a favorable change in MSC invasion capacity, proliferation, gene expression, ECM attachment, and VEGF secretion with subsequent increased angiogenesis. MSC, minocycline, vancomycin, and linezolid-loaded hydrogels decrease bacterial bioburden and accelerate healing in bacteria-inoculated wounds, demonstrating potential clinical viability for infection prophylaxis and advanced wound healing for full thickness cutaneous wounds.

References: Caplan AI. J Pathol. 2009;217:318-324. Xu K. Acta Biomater. 2013;9:8802-8814. Busscher HJ. Sci Transl Med. 2012;4:153rv10. Trivedi P. Exp Hematol. 2008;36:350-359.