Generation of a Novel Scaffold for In Vivo Polarization of Therapeutic Macrophages and Delivery of IL-10 to Improve Cutaneous Wound Healing

Leandra A Barnes, AB; Michael S Hu, MD, MPH, MS; Alexander TM Cheung; Arita Dubnika, PhD; Clement D Marshall, MD; Tripp Leavitt, BA, BS; Mohammed Ahmed, PhD; Geoffrey C Gurtner, MD; H Peter Lorenz, MD; Jayakumar Rajadas, PhD; Michael T Longaker, MD, MBA

Disclosure/Financial Support: Supported by the Howard Hughes Medical Institute Medical Research Fellowship, the California Institute for Regenerative Medicine (CIRM) Clinical Fellow training grant TG2-01159, the American College of Surgeons (ACS) Resident Research Scholarship, the American Society of Maxillofacial Surgeons (ASMS)/Maxillofacial Surgeons Foundation (MSF) Research Grant Award, NIH grant R01 GM087609, the Hagey Laboratory for Pediatric Regenerative Medicine and The Oak Foundation, and the Gunn/Olivier fund. None of the authors has a financial interest in any of the products, devices, or drugs mentioned in this manuscript.

INTRODUCTION: Defective cutaneous wound healing poses a significant clinical challenge, so it is necessary to develop novel techniques to improve tissue regeneration and repair. Macrophages are critical for effective wound repair, as their deletion slows healing. Specifically, M2 phenotype macrophages are pro-regenerative. Herein, we develop a novel scaffold to polarize macrophages to the M2 phenotype and examine their ability to improve wound healing *in vivo*.

METHODS: We developed a proprietary scaffold made from heparin and collagen containing recombinant interleukin-10 (IL-10). To characterize the release profile of IL-10 from the scaffolds, we indirectly assessed the amount of IL-10 in solution at ten time points using enzyme-linked immunosorbent assays (ELISA). The absorptive properties of the scaffold were determined by swelling studies in phosphate-buffered saline (PBS) at 37ºC. Surface microstructures of the scaffolds after being cross-linked with glutaraldehyde were observed under scanning electron microscope (SEM). Monocytes isolated from the bone marrow of mice were differentiated into macrophages after 7 days in culture with macrophage colony-stimulating factor (M-CSF) and seeded onto hydrated IL-10 scaffolds. Scaffolds were then delivered onto full-thickness splinted excisional wounds in mice to be polarized *in vivo*. We assessed wound size and healing progression with digital photographs. After wound closure, tissue was harvested for histologic, gene expression, and cellular analyses.

RESULTS: The release profile demonstrated peak release of 35.5 ng/ul of IL-10 at 4 hours with a concentration of at least 18.7 ng/ul IL-10 maintained for 48 hours. The scaffolds reached full swelling in PBS within 15 minutes with a swelling ratio of 16.53. Gene expression analysis confirmed that macrophages seeded onto IL-10 scaffolds were polarized to the M2 phenotype *in vitro* and *in vivo*. Full-thickness excisional wounds all healed significantly faster ($*p<0.05$) when treated with macrophages seeded onto the IL-10 scaffold.

CONCLUSION: Our results demonstrate that our novel proprietary scaffold can polarize macrophages to the M2 phenotype and deliver supra-physiologic levels of macrophages to wounds to significantly accelerate wound healing in the absence of adverse effects on scar size and quality. Polarizing macrophages on a scaffold *in vivo* minimizes time in cell culture and is a desirable method for simultaneous directed differentiation and cell delivery. With further studies, this could prove to be a novel therapeutic for wound regeneration.