Online Supplemental Information

Enhanced Potency of Cell-based Therapy for Ischemic Tissue Repair Using an Injectable Bioactive Epitope-presenting Nanofiber Support Matrix

Jörn Tongers, MD*[1,4]; Matthew J. Webber, PhD*[2]; Erin E Vaughan, PhD[1]; Eduard Sleep, PhD[2]; Marie-Ange Renault, PhD[1]; Jerome G. Roncalli, MD[1]; Ekaterina Klyachko[1], PhD; Tina Thorne[1]; Yang Yu, MD, PhD[1]; Katja-Theres Marquardt[1,4]; Christine E. Kamide[1]; Aiko Ito[1]; Sol Misener[1]; Meredith Millay[1]; Ting Liu[1]; Kentaro Jujo, MD[1]; Gangjian Qin MD PhD[1]; Douglas W. Losordo, MD[1]; Samuel I. Stupp, PhD[2,3#]; Raj Kishore, PhD[1,5#]

* JT and MJW contributed equally to this work

[1]- Feinberg Cardiovascular Research Institute, Feinberg School of Medicine, Northwestern University, Chicago, IL

[2]- Institute for Bionanotechnology In Medicine, Department of Medicine, Northwestern University, Chicago, IL

[3]- Department of Materials Science and Engineering, Department of Chemistry, Northwestern University, Evanston, IL

[4]- Department of Cardiology and Angiology, Hannover Medical School, Hannover, Germany

[5]-Center for Translational Medicine, Temple University School of Medicine, Philadelphia, PA

[#]-Address correspondence to:

Dr. Raj Kishore, PhD
Center for Translational Medicine
Department of Pharmacology
Temple University School of Medicine
MERB-953
3500 N Broad Street, Philadelphia, PA 19140
Phone: 215-707-2523
FAX: 215-707-9890
Raj.kishore@temple.edu

Prof. Samuel I. Stupp, PhD
Northwestern University
Cook Hall, Room 1127
2220 Campus Drive
Evanston, IL 60208, USA
Phone: (847)491-3002
Fax: (847)491-3010
s-stupp@northwestern.edu

Supplemental Results:

RGDS-PA nanofibers support human-derived CD34⁺ cell therapy

Although widely used in the clinical setting, bone marrow-derived cells constitute an unselected population. To further evaluate the impact of the RGDS-PA matrix on a selected, well-characterized population, human CD34⁺ cells were employed along with the RGDS-PA matrix in the HLI model. Similar to the findings with BMPACs, significant improvements in blood flow, limb salvage, and motor function were observed for CD34⁺ cells injected with RGDS-PA when compared to treatment with CD34⁺ cells alone (***Figure S1***). This suggests a versatile role for RGDS-PA to support and improve therapies for ischemic tissue based on various stem or progenitor cell populations.

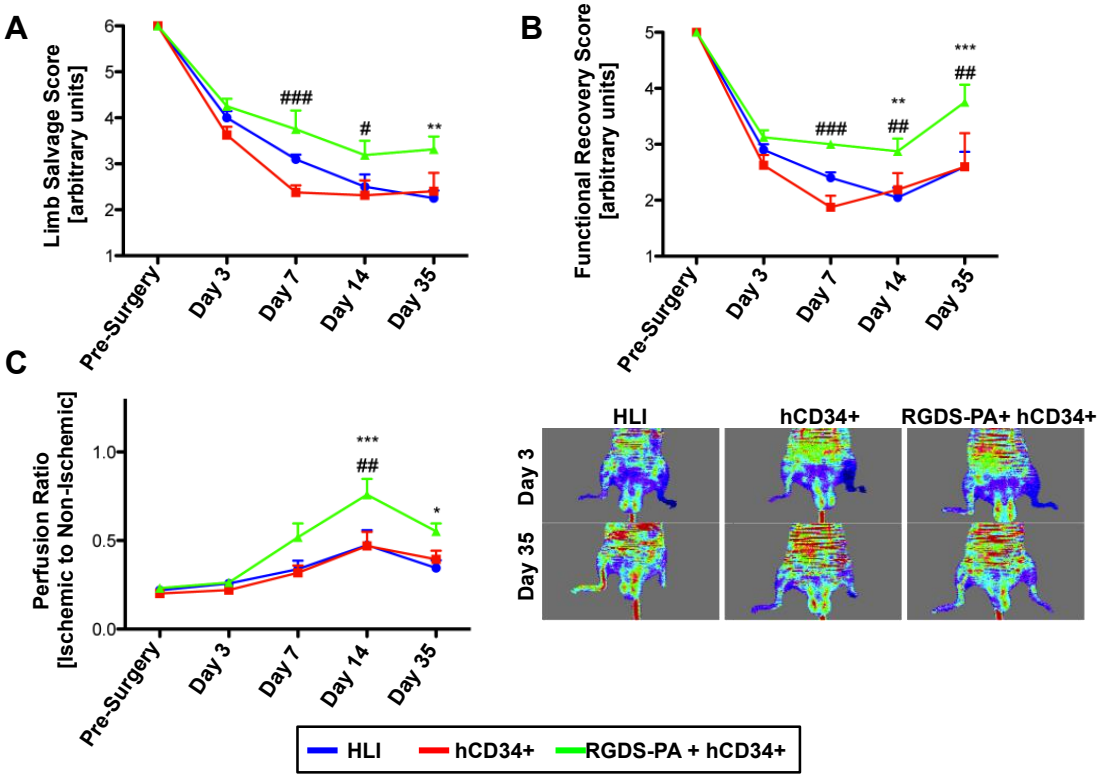

Figure S1. *In vivo* evaluation of hCD34⁺ cells delivered with RGDS-PA. **(A)** Limb salvage score for each group at each time point from mice injected with PBS (HLI), hCD34⁺ cells (hCD34+) or hCD34+ cells with RGDS-PA (RGDS-PA+hCD34⁺). **(B)** Limb motor function score for each treatment. **(C)** Normalized perfusion ratios from LDPI at each timepoint of the study, along with representative LDPI images from day 3 (when treatment was injected) and day 35. P<0.05, P<0.01, P<0.001 correspond to *, **, ***, and #, ##, ### for RGDS-PA+hCD34⁺ vs HLI, and RGDS-PA+hCD34⁺ vs. hCD34⁺, respectively.

Biocompatibility and tissue stability of RGDS-PA

An important factor for the clinical translation of biomaterials is the immune response that might be elicited by their presence in tissue. There was no evidence of a systemic inflammatory response in a peripheral blood count (***Figure S2A***) following intramuscular RGDS-PA injection at 2 (acute), 7 (subacute), or 28 (chronic) days following administration. Leukocyte number, a marker of inflammation/infection, was similar in RGDS-PA treated animals compared to controls at all time points. Additionally, no changes were observed for any leukocyte subpopulations following administration of RGDS-PA. Since a systemic response would be expected only for severe incompatibility, we looked at the local cellular response after injection of PA into the limb muscle. Evaluating sequential sections of gastrocnemius muscle by histology, we observed no increase in total infiltrating cells, nor a specific increase in $CD45^+$ (i.e. leucocytes) or $CD68^+$ cells (i.e. macrophages). To sample the response in the entire tissue, we quantified inflammatory cells from the whole muscle by FACS-analysis, and saw no significant increase in the number of $CD45^+$ (leucocytes) or $F4/80^+$ (macrophages) cells in the tissue (***Figure S2B***). Since PA nanofibers degrade over time, we wanted to determine how long RGDS-PA remained in the limb. By means of a vigorous histological search, we were able to detect PA matrix up to 28 days in a non-ischemic limb, but only up to 14 in the ischemic limb. This suggests that the milieu of ischemic tissue may result in more rapid PA degradation.

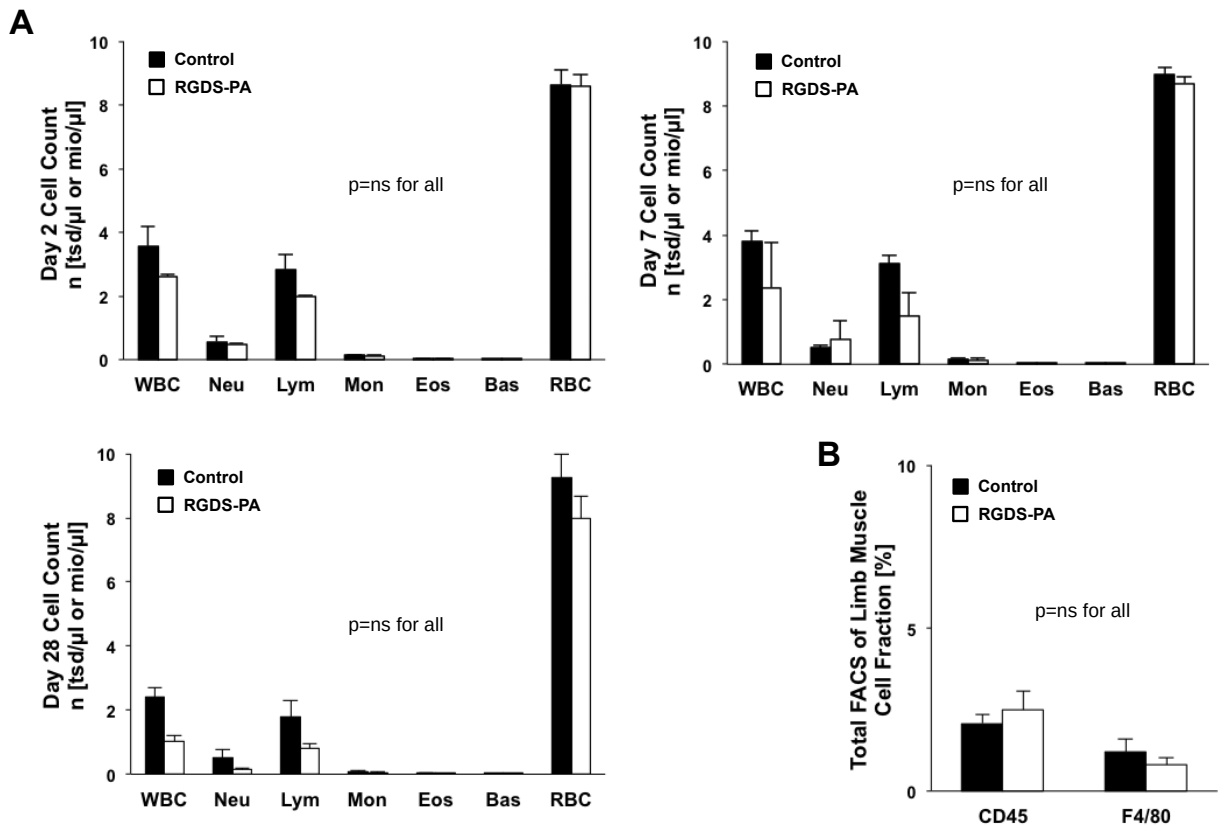

Figure S2. (A) A peripheral blood count for systemic immune response performed at day 2 (acute phase), 7 (sub acute phase) and 28 (chronic phase). **(B)** The local inflammatory response following intramuscular RGDS-PA injection, determined by FACS analysis of the whole gastronemius muscle stained for $CD45^{+}$ leucocytes and F4/80 macrophages.