

Supplementary Materials for

Osteogenic preconditioning in perfusion bioreactors improves vascularization and bone formation by human bone marrow aspirates

J. N. Harvestine, T. Gonzalez-Fernandez, A. Sebastian, N. R. Hum, D. C. Genetos, G. G. Loots, J. K. Leach*

*Corresponding author. Email: jkleach@ucdavis.edu

Published 12 February 2020, *Sci. Adv.* **6**, eaay2387 (2020)
DOI: 10.1126/sciadv.aay2387

The PDF file includes:

Fig. S1. Genes associated with immune system development are down-regulated with increasing time in culture.

Fig. S2. HA is degraded and removed from composite scaffolds during preconditioning in the bioreactor.

Fig. S3. Preconditioned scaffolds exhibit improved integration with host bone.

Legends for tables S1 to S3

Other Supplementary Material for this manuscript includes the following:

(available at advances.sciencemag.org/cgi/content/full/6/7/eaay2387/DC1)

Table S1 (Microsoft Excel format). Genes associated with immune system development down-regulated in preconditioned compared to naïve constructs.

Table S2 (Microsoft Excel format). Genes associated with osteoblast differentiation and bone development up-regulated in preconditioned compared to naïve constructs.

Table S3 (Microsoft Excel format). Genes associated with vasculature development up-regulated in preconditioned compared to naïve constructs.

Supplementary Figures and Tables

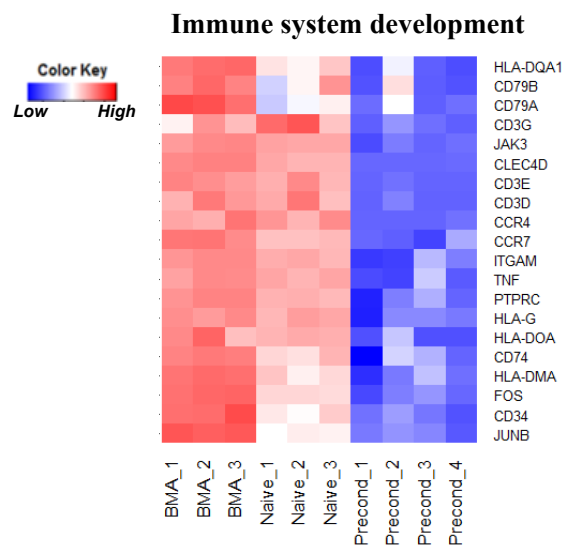


Fig. S1. Genes associated with immune system development are down-regulated with increasing time in culture. Heatmap of RNA-seq data for 20 key genes associated with immune system development (n=3-4).

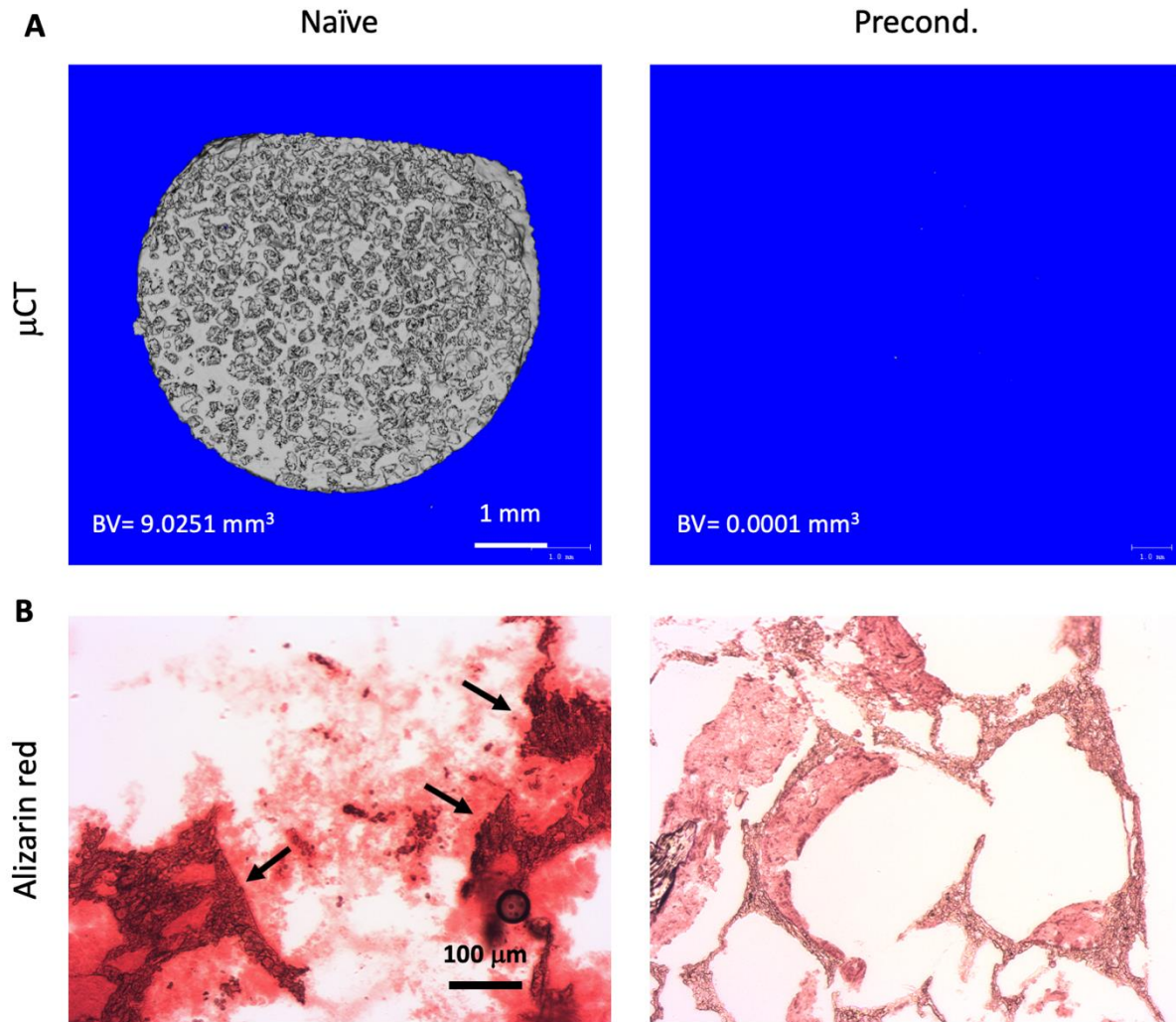


Fig. S2. HA is degraded and removed from composite scaffolds during preconditioning in the bioreactor. Mineralization assessment of the naïve and preconditioned implants before *in vivo* implantation. **(A)** microCT analysis and bone volume in the naïve and preconditioned groups. **(B)** Alizarin red staining of the calcium present on the samples. Black arrows denote intense positive calcium staining.

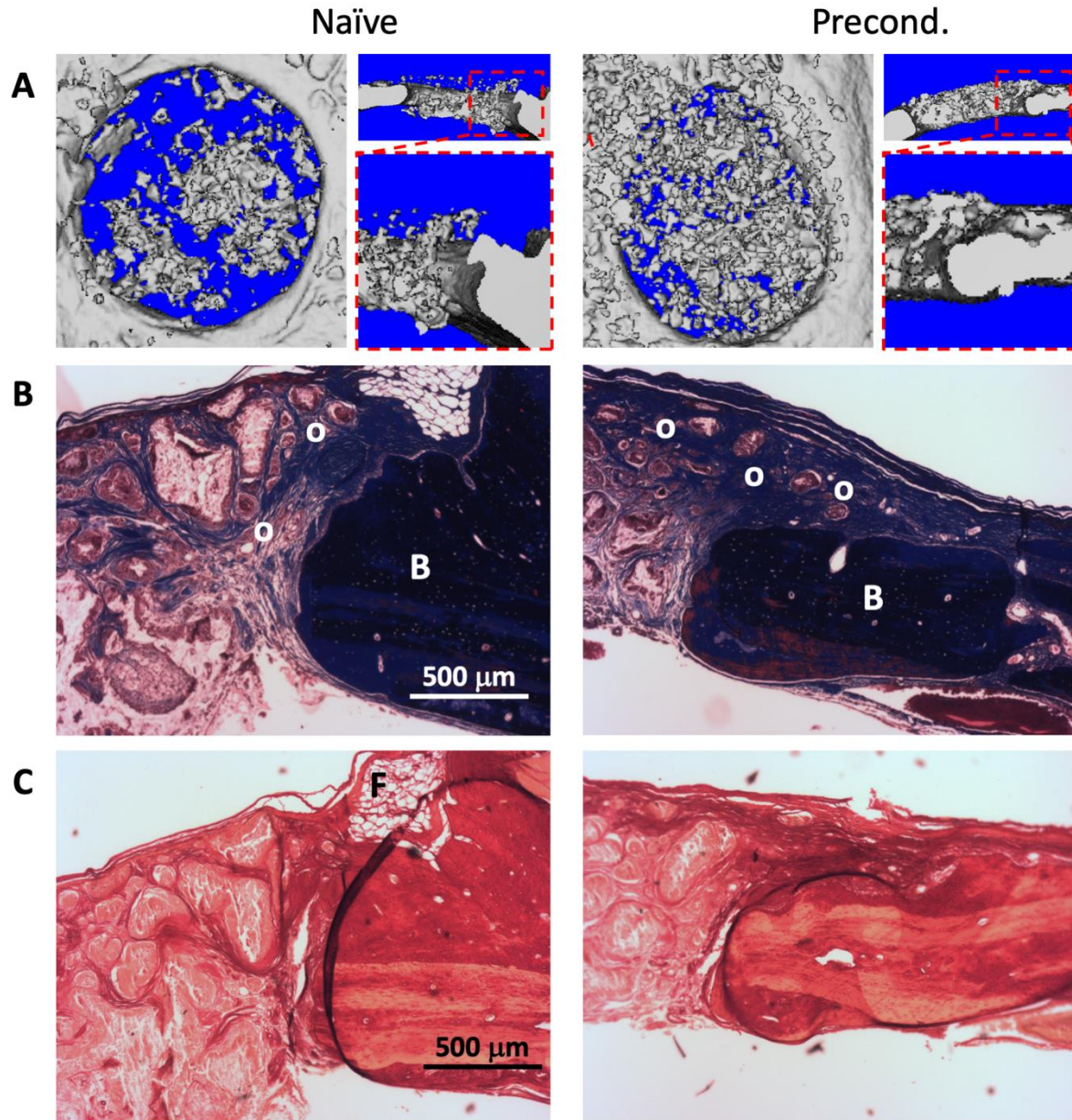


Fig. S3. Preconditioned scaffolds exhibit improved integration with host bone. Assessment of new bone integration at the margins of the defects treated with either naïve or preconditioned constructs at week 10 after implantation. (A) Representative microCT analysis in the naïve and preconditioned groups. (B) Representative Masson's trichrome and (C) Picrosirius red staining of the new bone and collagen content of the treated bone defects. "B" indicates native bone areas, "o" indicates areas of osteoid formation and "F" indicates areas of fibrous tissue.

Table S1. Genes associated with immune system development down-regulated in preconditioned compared to naïve constructs. All the genes have a fold change value < 2 (\log_2 fold change < 1) and FDR adjusted p -value < 0.01 .

Table S2. Genes associated with osteoblast differentiation and bone development up-regulated in preconditioned compared to naïve constructs. All the genes have a fold change value > 2 (\log_2 fold change > 1) and FDR adjusted p -value < 0.01 .

Table S3. Genes associated with vasculature development up-regulated in preconditioned compared to naïve constructs. All the genes have a fold change value > 2 (\log_2 fold change > 1) and FDR adjusted p -value < 0.01 .