# Drug Free ROS Sponge Polymeric Microspheres Reduce Tissue Damage from Ischemic and Mechanical Injury

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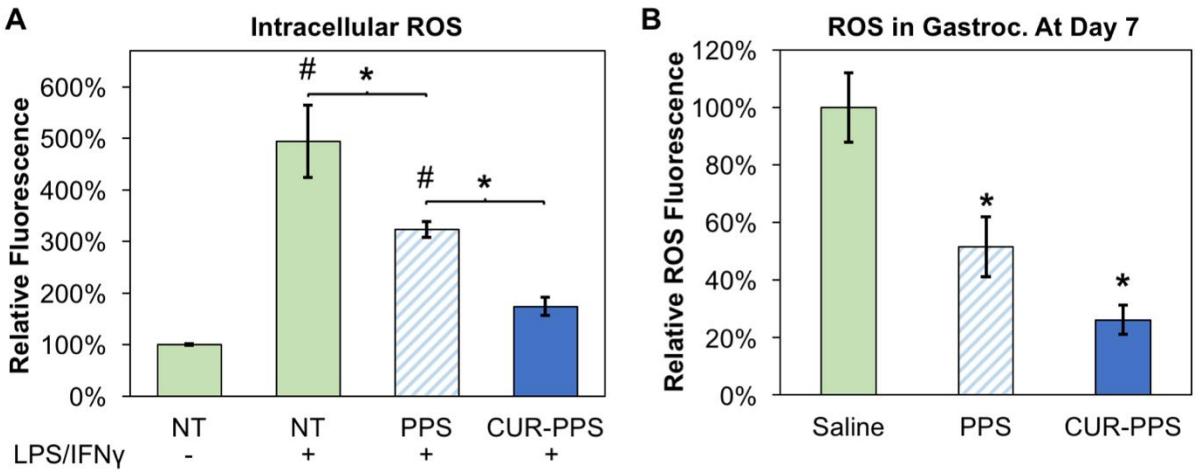


**Figure S.1: Curcumin-loaded and blank PPS microspheres exhibit antioxidant activity *in vitro* and *in vivo*. A) Intracellular ROS levels are reduced in LPS/IFN-γ-stimulated RAW macrophages by treatment with PPS microspheres and CUR-PPS microspheres ($p < 0.05$). Intracellular ROS levels in activated macrophages treated with CUR-PPS microspheres were statistically equivalent to the non-activated RAW cells. Microsphere doses contain 3.4 μM curcumin or the equivalent polymer dose. *$p < 0.05$ for differences between indicated groups. #$p < 0.05$ relative to unstimulated macrophages (LPS/IFN-γ(−)/NT group). B) Blank PPS microspheres and curcumin-loaded PPS microspheres significantly reduce ROS in gastrocnemius muscles extracted from ischemic limbs. Data presented as mean ± SEM. Saline group $n = 8$, blank PPS group $n = 11$, curcumin-PPS group $n = 10$. *$p < 0.05$ relative to saline treatment. Reproduced with permission from Biomaterials, 41 (0). Poole, K.M., et al. *ROS-responsive microspheres for on demand antioxidant therapy in a model of diabetic peripheral arterial disease.* 166-175. Copyright 2015 Elsevier.[1]**

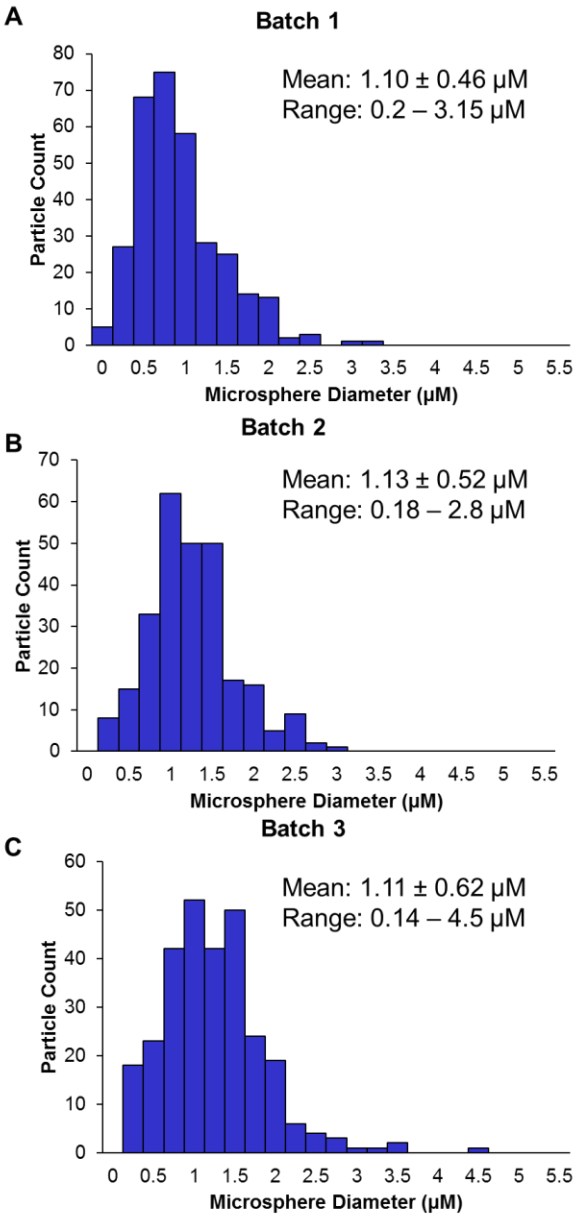


**Figure S.2: Characterization of PPS-MS across multiple batches. The size distribution of the PPS-MS was measured with SEM, and the mean diameter and diameter range were (A) 1.10 ± 0.46 µm and 0.2 – 3.15 µm, (B) 1.13 ± 0.52 µm and 0.18 – 2.8 µm, and (C) 1.11 ± 0.62 µm and 0.14 – 4.5 µm, respectively.**

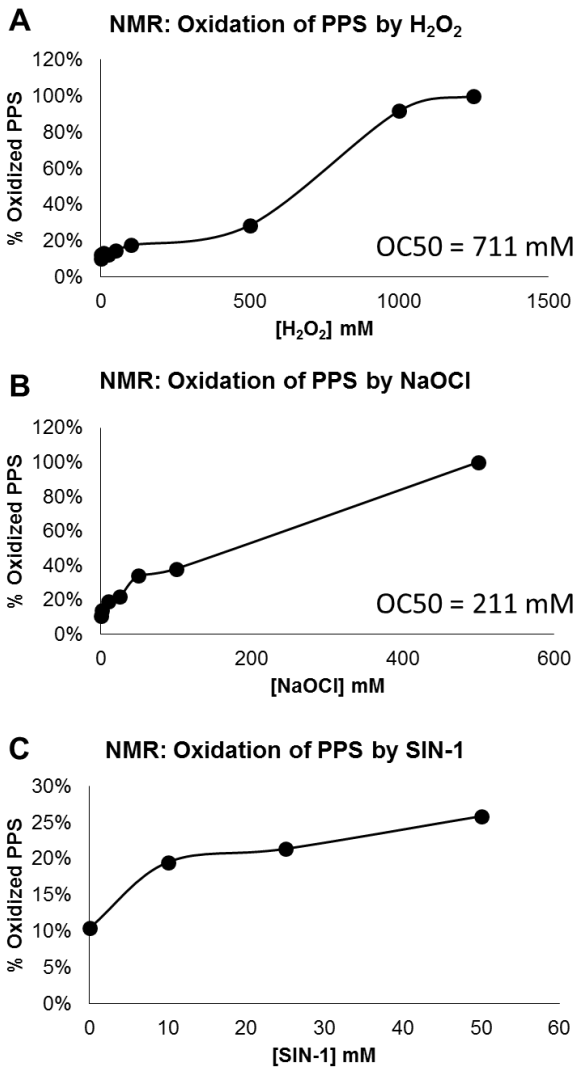


**Figure S.3: PPS is oxidized at different rates by various ROS. PPS was incubated with various ROS for 24 hours, lyophilized, dissolved in $CDCl_3$, and run on $^1H$ NMR to detect PPS and oxidized PPS. (A) $H_2O_2$ oxidizes PPS with an OC50 = 711 mM. (B) NaOCl oxidizes PPS with an OC50 = 211 mM. (C) SIN-1 (50 mM) causes approximately 15% PPS oxidation over baseline.**

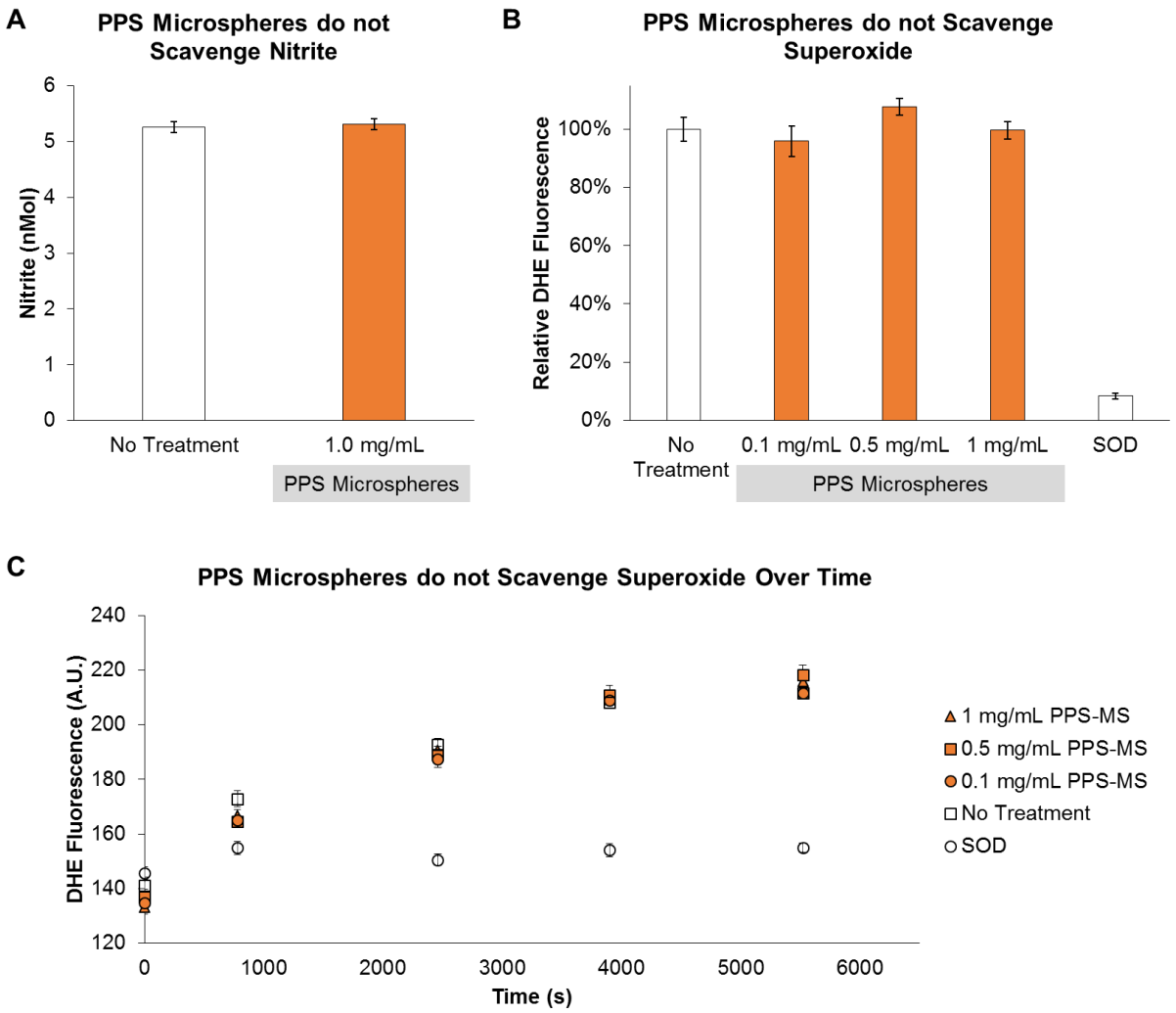


**Figure S.4: PPS-MS do not scavenge nitrite and superoxide. A) PPS-MS do not scavenge nitrite after 1 hour incubation. B) PPS-MS do not scavenge superoxide produced by the X/XO system after 1 hour of incubation. C) PPS-MS do not scavenge superoxide produced by the X/XO system over time.**

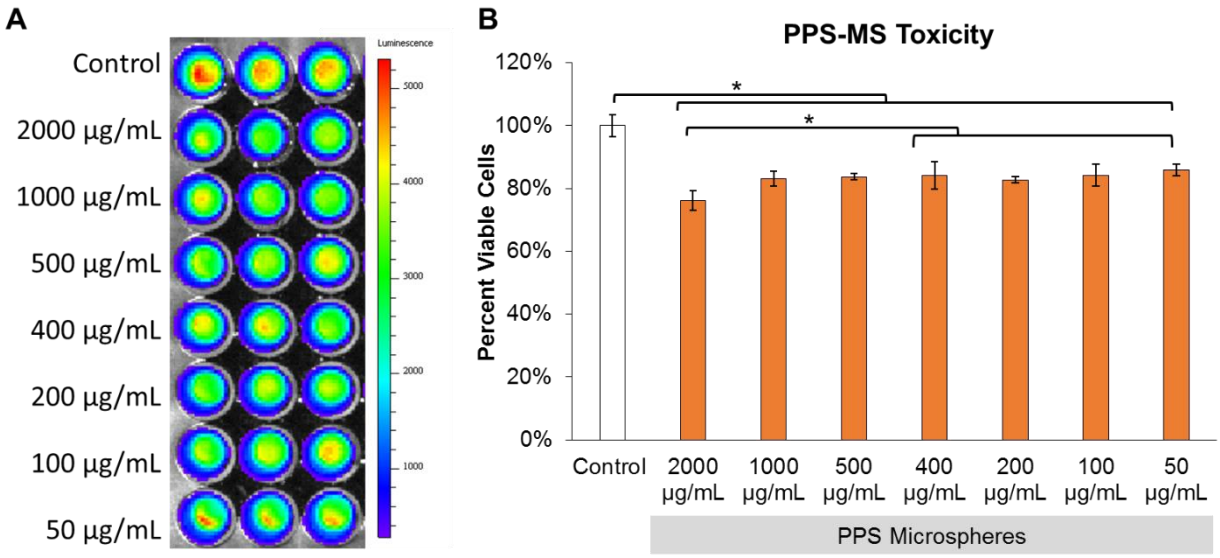


**Figure S.5: PPS-MS are cytocompatible at high doses. Cell viability is greater than 80% in RAW 264.7 macrophages treated with PPS-MS at doses less than 1000 µg/mL (doses used for ROS assays were 400-50 µg/mL). A) Representative luminescent image of CellTiter-Glo Assay. B) Quantified luminescence of PPS-MS treated cells for cell viability (one-way ANOVA $p<0.05$, *significant post-hoc comparisons).**

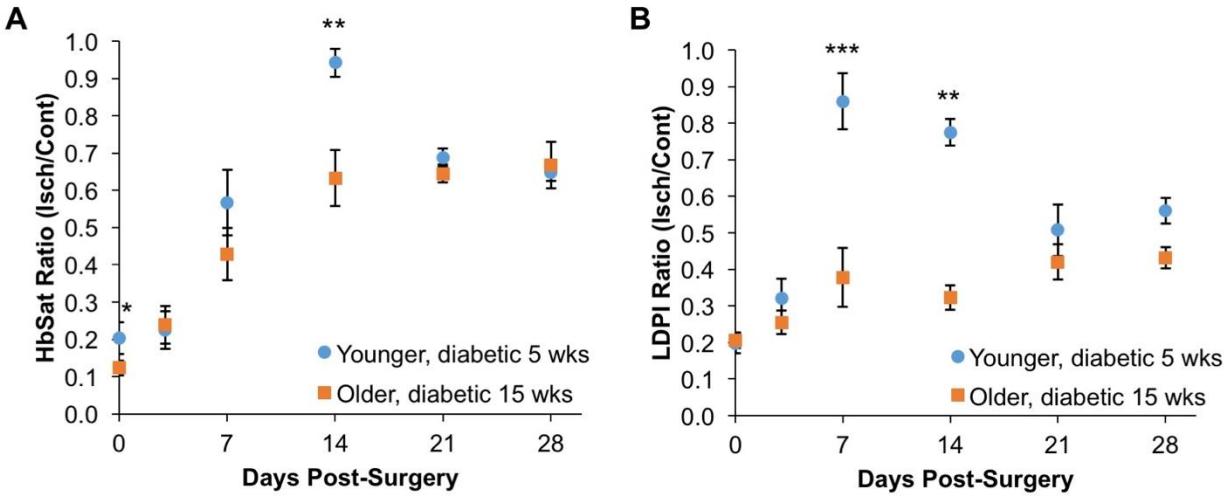


**Figure S.6: Younger diabetic mice exhibit an early "overshoot" response in distal measures of vascular recovery that is absent from the response to ischemia in older diabetic mice. n=5/group for younger cohort and n≥6/group for older cohort for HbSat (A) and perfusion measurements (B). **p<0.01, ***p<0.001 for rank sum test between younger and older cohort at a given time point.**

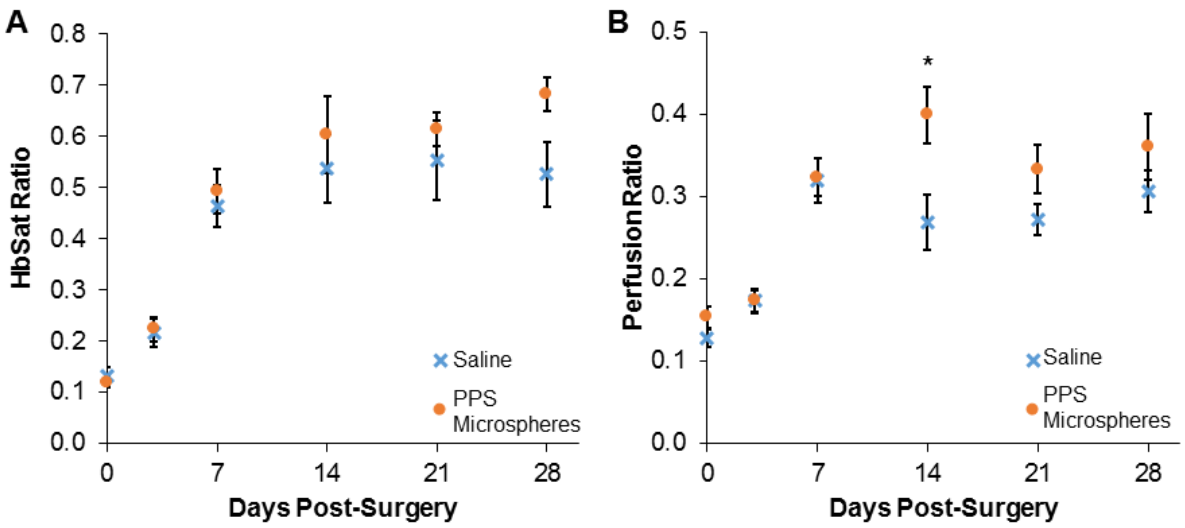


**Figure S.7: PPS microspheres improve functional vascular recovery from hind limb ischemia. HbSat and perfusion were measured in the footpads of diabetic mice treated with saline or PPS microspheres. A) Individual time point analyses did not identify significant differences between treatment groups in the HbSat response. B) At day 14, perfusion in the PPS group was significantly greater than that in the saline group ($p<0.05$). n=17-20/group for days 0-7 and n=6-7/group for days 14-28. *$p<0.05$.**

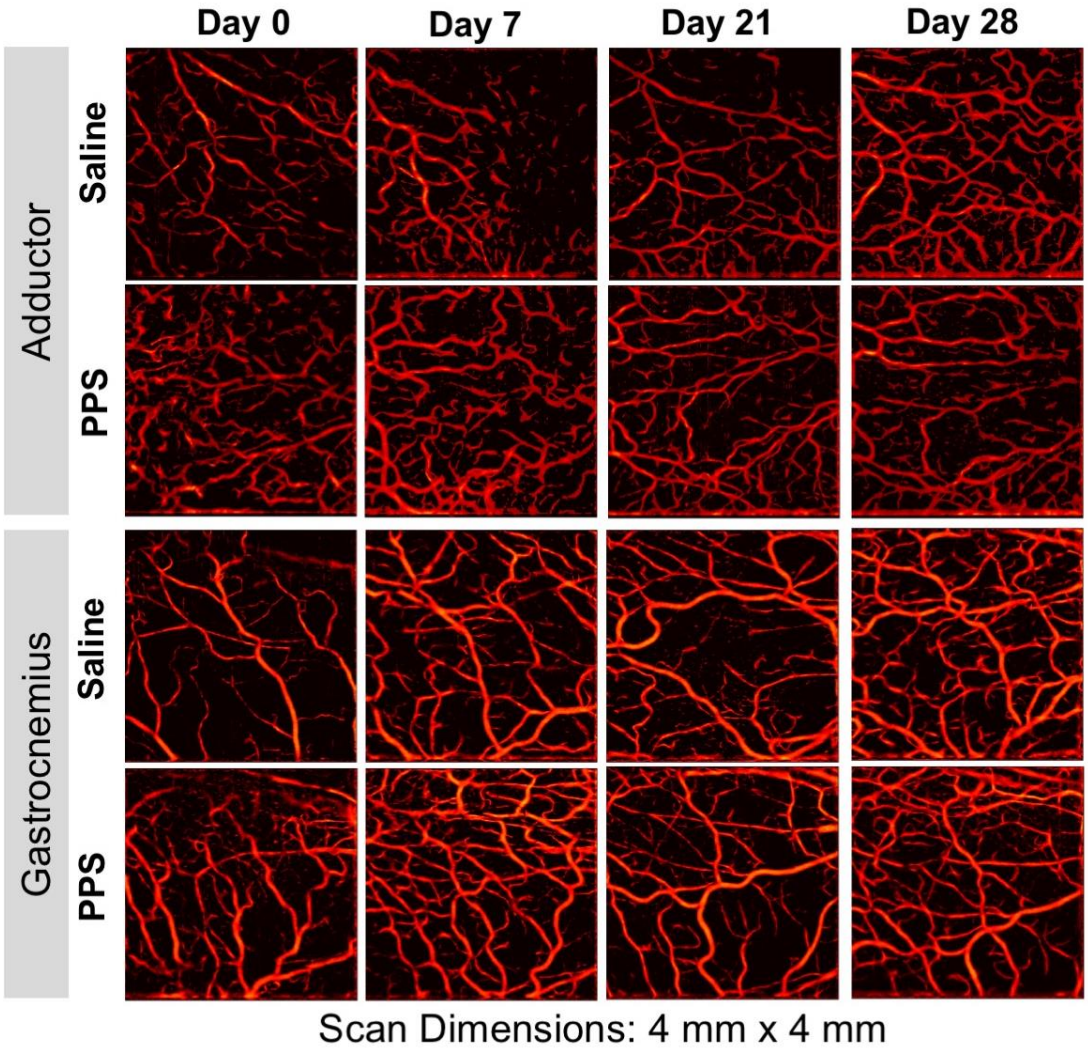


**Figure S.8: Representative time course images of vascular morphology in the adductor and gastrocnemius muscle regions for each treatment group show that the vessel density differs between the two groups earlier in the time course, while the groups converge by the end of the time course. Images are projections of all vessels present in the volume acquired over a 4 mm x 4 mm area.**

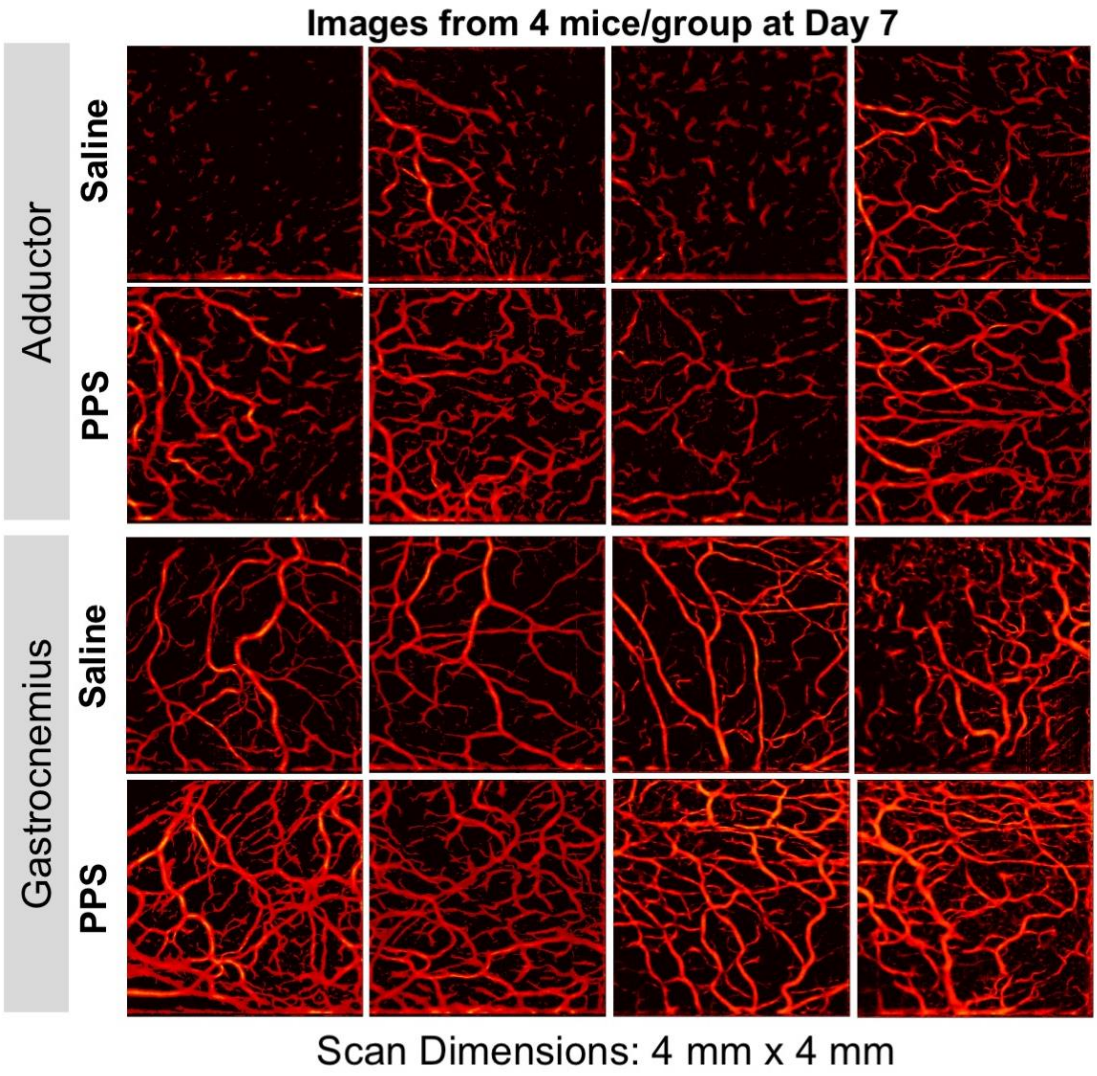


**Figure S.9: Representative images of vascular morphology in the adductor and gastrocnemius muscle regions for four mice in each treatment group show that PPS microsphere-treated mice have an increased vessel density at day 7. Images are projections of all vessels present in the volume acquired over a 4 mm x 4 mm area.**

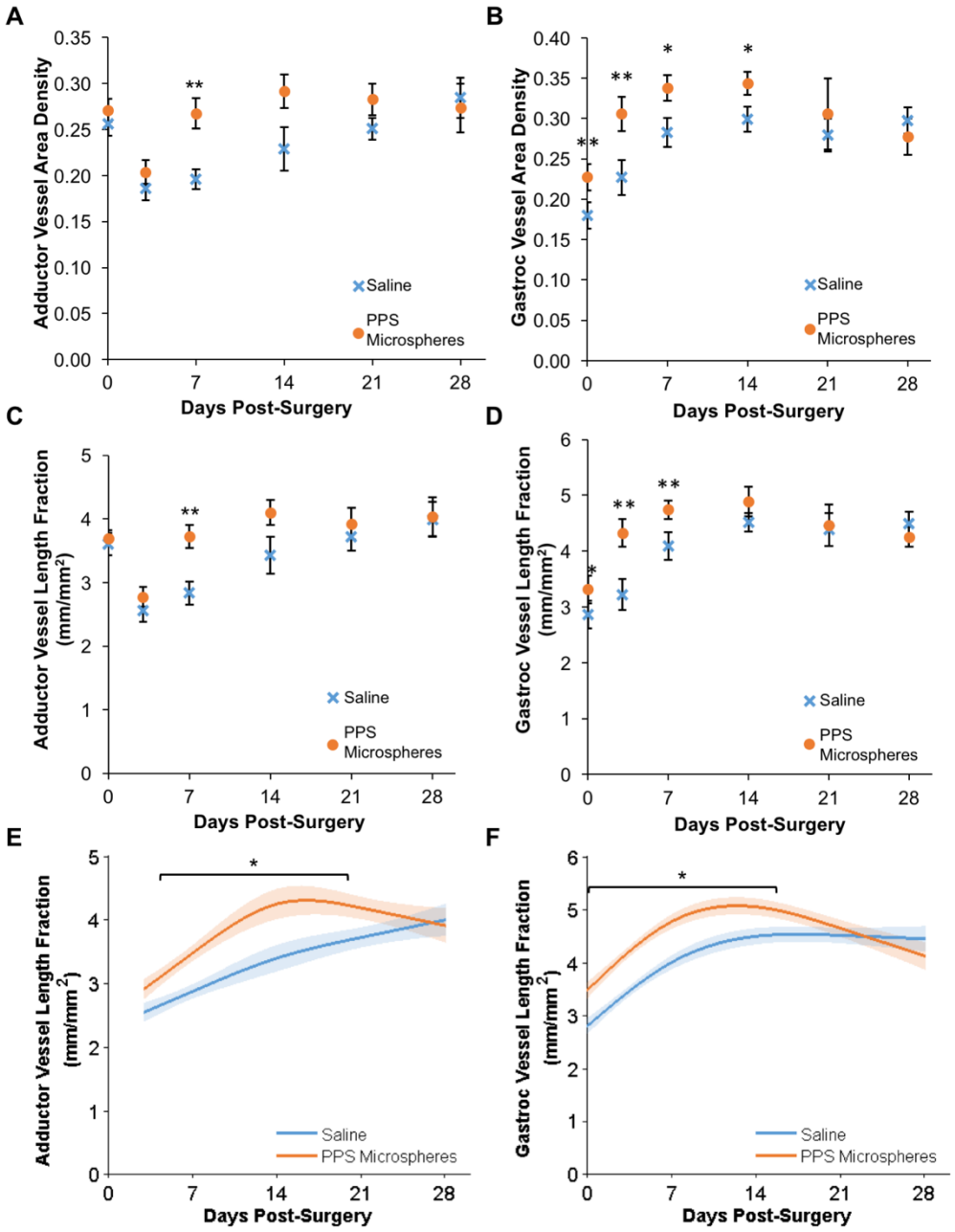


**Figure S.10: Vessel morphology parameters were quantified from OCT images of the adductor and gastrocnemius muscle regions. At day 7, vessel area density (A) was significantly lower in the adductor of the saline group than that in the PPS-MS-treated group. Vessel area density in the gastrocnemius (B) differed significantly at days 0, 3, 7, and 14 between the two groups. At day 7, vessel length fraction (C) was significantly lower in the adductor of the saline group than that in the PPS-MS group. Vessel length fraction in the gastrocnemius (D) differed significantly at days 0, 3, and 7 between the two groups. Analysis of the GLM curves for vessel length fraction shows that the two groups differ significantly from days 4-20 in the adductor (E) and from days 0-15 in the gastrocnemius (F). n=17-20/group for days 0-7 and n=6-7/group for days 14-28. *p<0.05, **p<0.01.**

**Supplemental Material References:**

1. Poole, K. M.; Nelson, C. E.; Joshi, R. V.; Martin, J. R.; Gupta, M. K.; Haws, S. C.; Kavanaugh, T. E.; Skala, M. C.; Duvall, C. L., ROS-responsive microspheres for on demand antioxidant therapy in a model of diabetic peripheral arterial disease. *Biomaterials* **2015,** *41* (0), 166-175.