Skeletal muscle regeneration can become permanently impaired by traumatic injuries, leading to volumetric muscle loss. Implantation of engineered biomimetic scaffolds to the site of muscle ablation may serve as an attractive off-the-shelf therapeutic approach. The objective of the study was to histologically assess the therapeutic benefit of a three-dimensional spatially patterned collagen scaffold, in conjunction with rehabilitative exercise, for treatment of volumetric muscle loss. To mimic the physiologic organization of skeletal muscle, which is generally composed of myofibers aligned in parallel, three-dimensional parallel-aligned nanofibrillar collagen scaffolds were fabricated. When implanted into the ablated murine tibialis anterior muscle, the aligned nanofibrillar scaffolds, in conjunction with voluntary caged wheel rehabilitative exercise, significantly improved the density of perfused microvessels, in comparison to treatments of the randomly oriented nanofibrillar scaffold, decellularized scaffold, or in the untreated control group. The abundance of neuromuscular junctions was 19-fold higher when treated with aligned nanofibrillar scaffolds in conjunction with exercise, in comparison to treatment of aligned scaffold without exercise. Although, the density of de novo myofibers was not significantly improved by aligned scaffolds, regardless of exercise activity, the cross-sectional area of regenerating myofibers was increased by >60% when treated with either aligned and randomly oriented scaffolds, in comparison to treatment of decellularized scaffold or untreated controls. These findings demonstrate that voluntary exercise improved the regenerative effect of aligned scaffolds by augmenting neurovascularization, and have important implications in the design of engineered biomimetic scaffolds for treatment of traumatic muscle injury.