Aligned and electrically conductive 3D collagen scaffolds for skeletal muscle tissue engineering

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Supplemental Figures

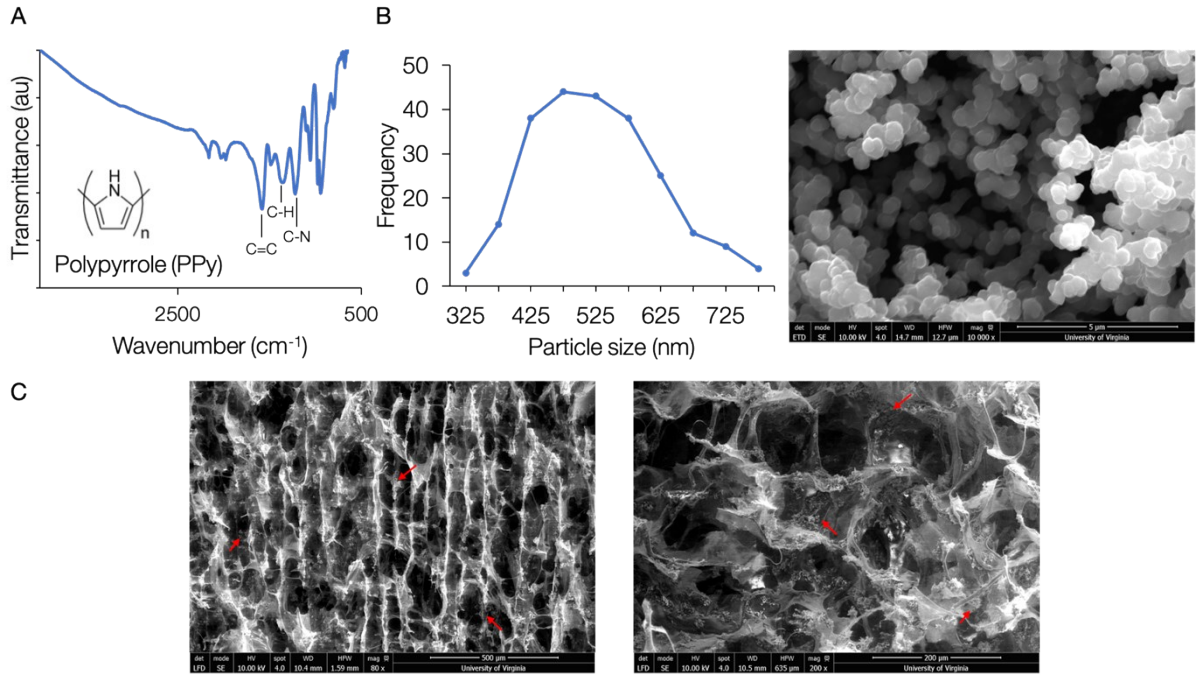

Figure S1. A) Polypyrrole (PPy) nanoparticles were synthesized via an oxidation reaction. FTIR analysis showed peaks at 1580 cm^{-1} associated with C=C stretching and peaks at 1350 cm^{-1} and 1220 cm^{-1} that are indicative of C-H wagging vibrations and conjugated C-N in-plane stretching. B) Particle size analysis using scanning electron microscope (SEM) images indicated the production of homogeneous particles with an average diameter of 527.1 ± 96.7 nm. n = 230 PPy particles. C) PPy nanoparticles were incorporated into a collagen chondroitin sulfate suspension prior to lyophilization. SEM images of lyophilized scaffolds show uniform distribution of PPy particles (*red arrows*) throughout the scaffold with minimal particle aggregation.

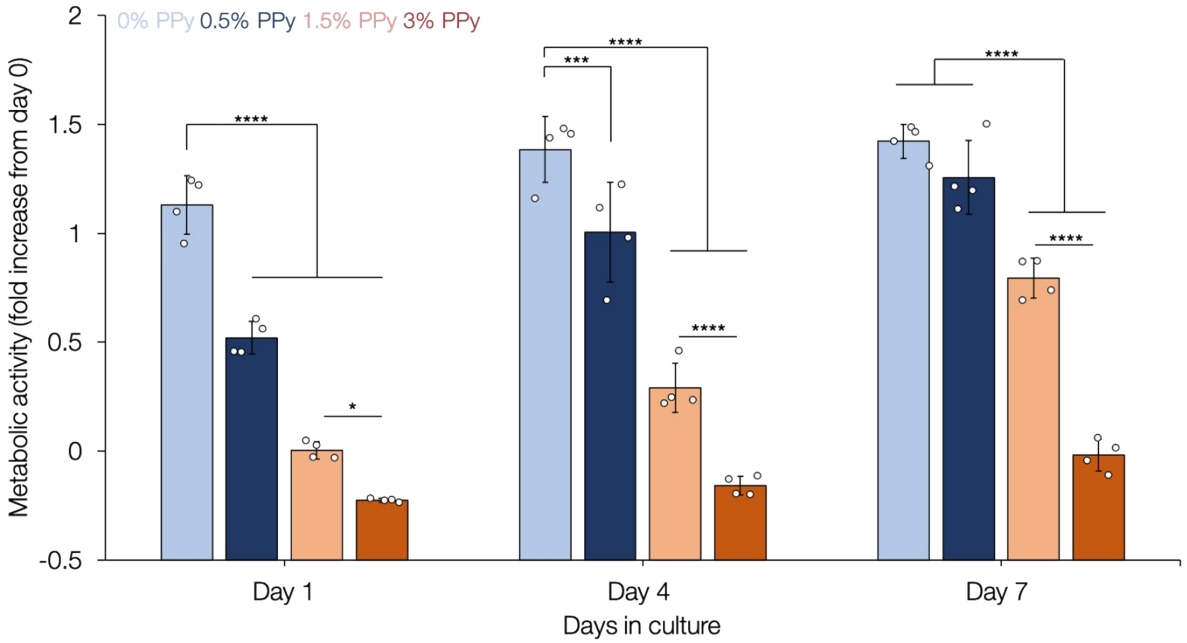

Figure S2. Quantification of metabolic activity over 7 days of culture showed that scaffolds with higher loading of polypyrrole (1.5, 3 wt%) promoted significantly decreased C2C12 myoblast metabolic activity. *: $P < 0.05$, ***: $P < 0.001$, ****: $P < 0.0001$. $n = 4$ scaffold per experimental group.

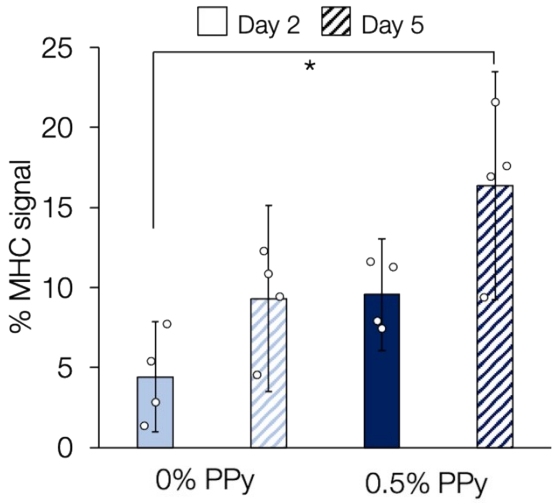

Figure S3. The percentage of the image area occupied by myosin heavy chain (MHC) staining was quantified using ImageJ. After 5 days in differentiation media there was significantly higher signal in the PPy-containing group compared to day 2 signal for the CG-only scaffold control. *: $P < 0.05$. $n = 4$ scaffolds per experimental group.

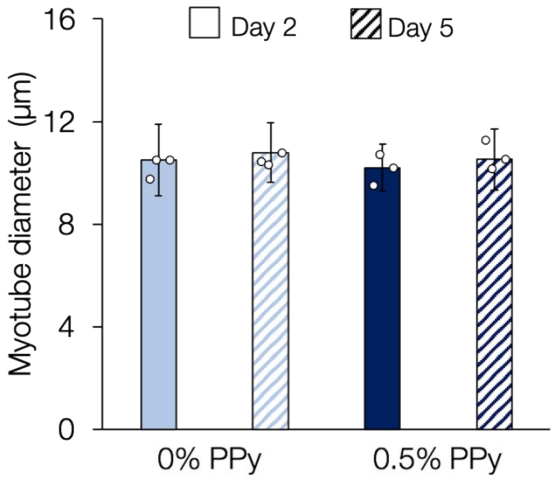

Figure S4. Myotube diameter was quantified using DiameterJ, an ImageJ plugin. There were no significant changes observed in myotube diameter as a function of culture time or PPy incorporation. $n = 3$ scaffolds per experimental group.

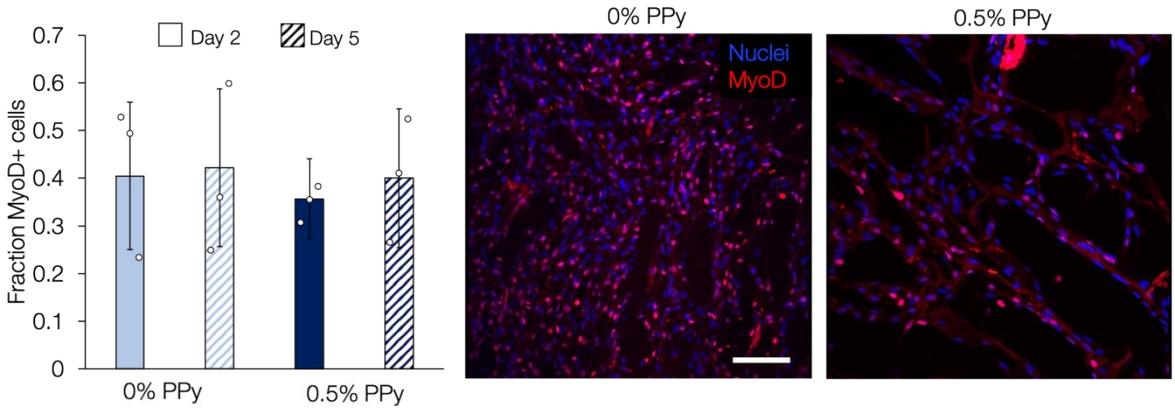

Figure S5. Assessment of myogenic differentiation indicated that culture time and PPy incorporation did not appreciably affect MyoD expression. *Scale bar:* 100 µm. $n = 3$ scaffolds per experimental group.

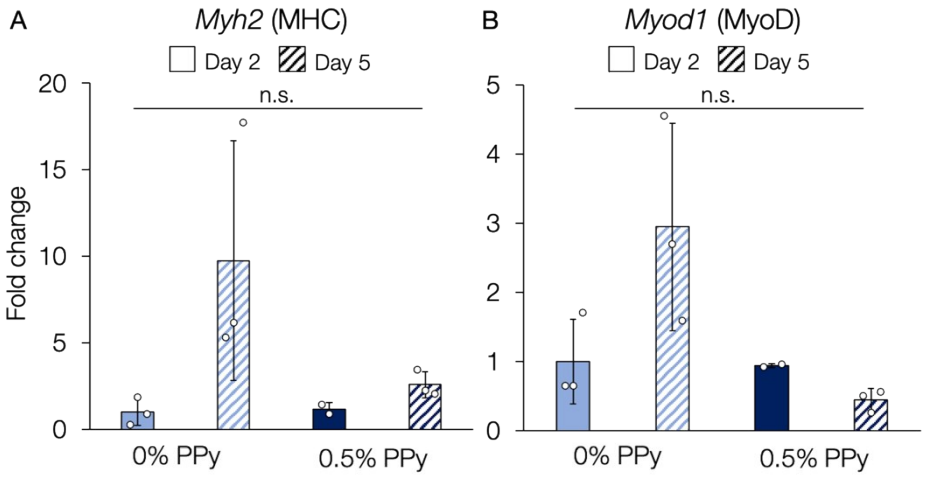

Figure S6. Expression of the A) *Myh2* and B) *Myod1* genes encoding for MHC and MyoD respectively were quantified after 2 and 5 days of culture in differentiation media for the 0 and 0.5 wt% PPy scaffolds. No statistically significant differences were found between any of the experimental groups. Data are expressed as the mean fold change normalized to expression in the 0% PPy day 2 group, $n = 3$ scaffolds per experimental group.