Supplementary Information (SI) for Journal of Materials Chemistry B.
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SUPPLEMENTARY INFORMATION

Fibers characterization

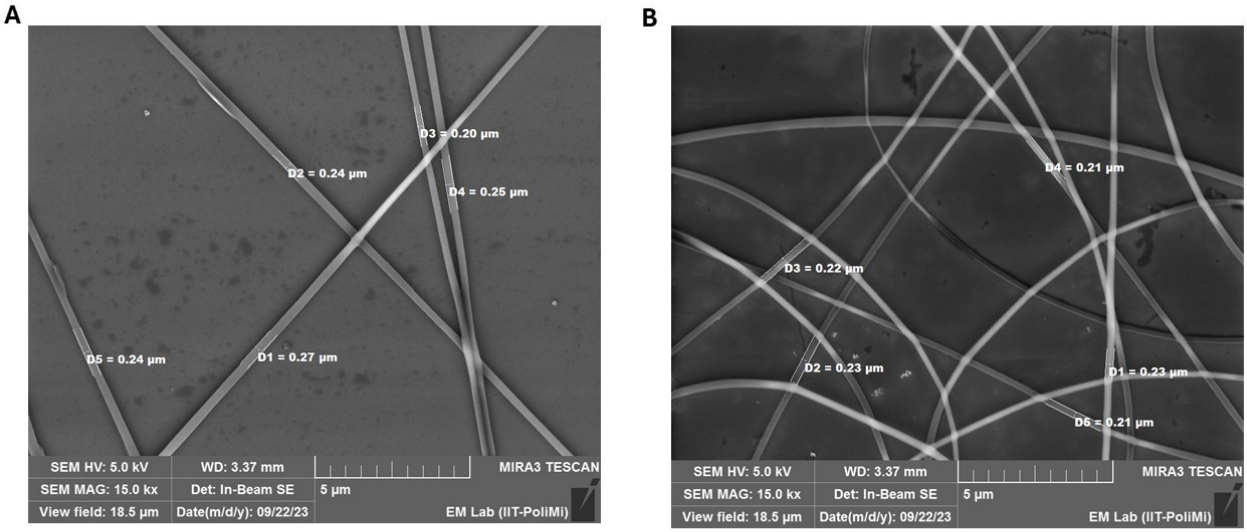


Figure S1. A) SEM image of PVA aligned nanofibers and diameters dimensions, B) SEM image of PVA random nanofibers and diameters dimensions

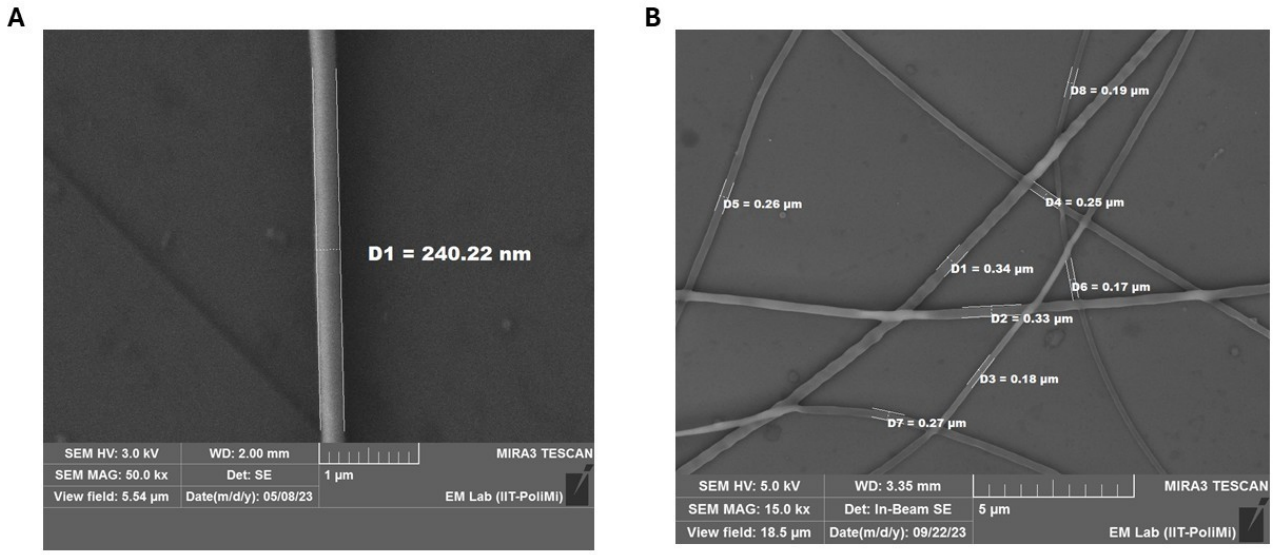


Figure S2. A) SEM image of PCL aligned nanofibers and diameters dimensions, B) SEM image of PCL random nanofibers and diameters dimensions.

Fusion index

Examples of confocal images used for the fusion index analysis are reported. In Figures A and B, aligned samples of PCL and PVA, respectively, are shown. In these samples, the myotubes exhibit longer structures and a higher number of nuclei compared to the corresponding randomly oriented nanofibrous samples. This observation indicates the importance of aligned substrates in promoting cell alignment and cell fusion, thereby favoring the differentiation process. To acquire these images, a 20× objective was used, and the cells were stained with Hoechst and CellMask deep red.

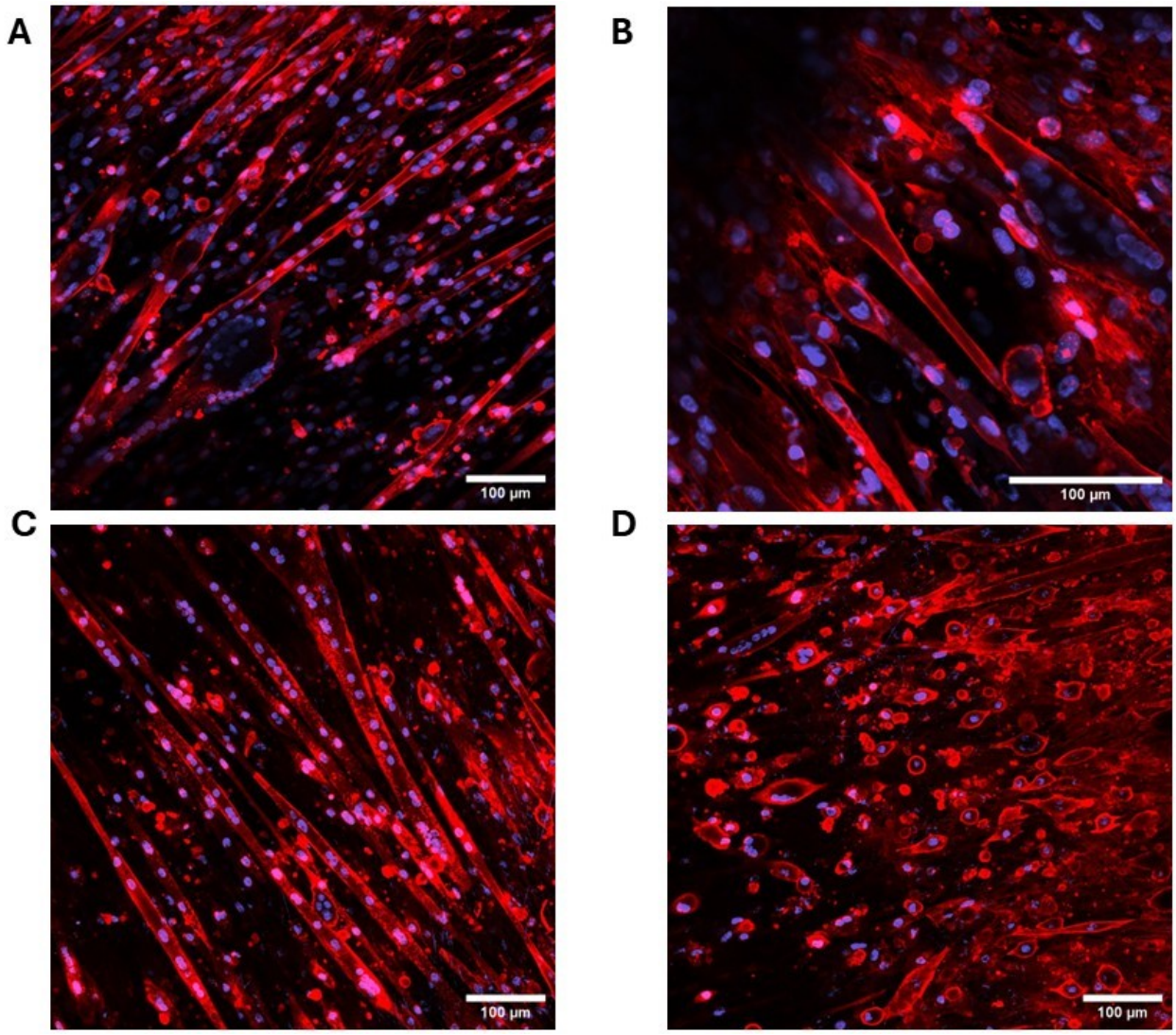


Figure S3. Confocal images of Myotubes after 12 days of differentiation on different substrates: A) PCL aligned, B) PCL random, C) PVA aligned and D) PVA random.

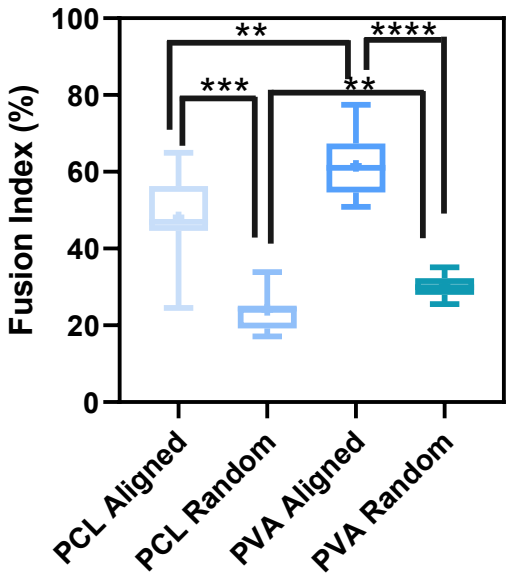


*Figure S4. Representative box plots of fusion index values (%) with standard deviation. A parametric t-student was performed for statistical analysis (** represents p = 0.018, *** p = 0.0006, **** p < 0.0001).*

Opto-stimulation and contraction analysis

During 1 Hz stimulation, approximately 68% of myotubes in aligned PVA followed the light stimulus perfectly, while at 2 Hz this dropped to about 30%. In PVA random samples, at 1 Hz and 2 Hz, roughly 60% and 35% of the myotubes contracted synchronously with the light pulses (Figure 3).
In PCL, as the stimulation frequency was increased, the ability of the cells to follow the stimulus decreased to about 50–60% (Figure 4). This performance deterioration may stem from poor mechanical properties of the cell seeded scaffold, or perhaps from the light stimulation process and the transducer molecular dynamics.

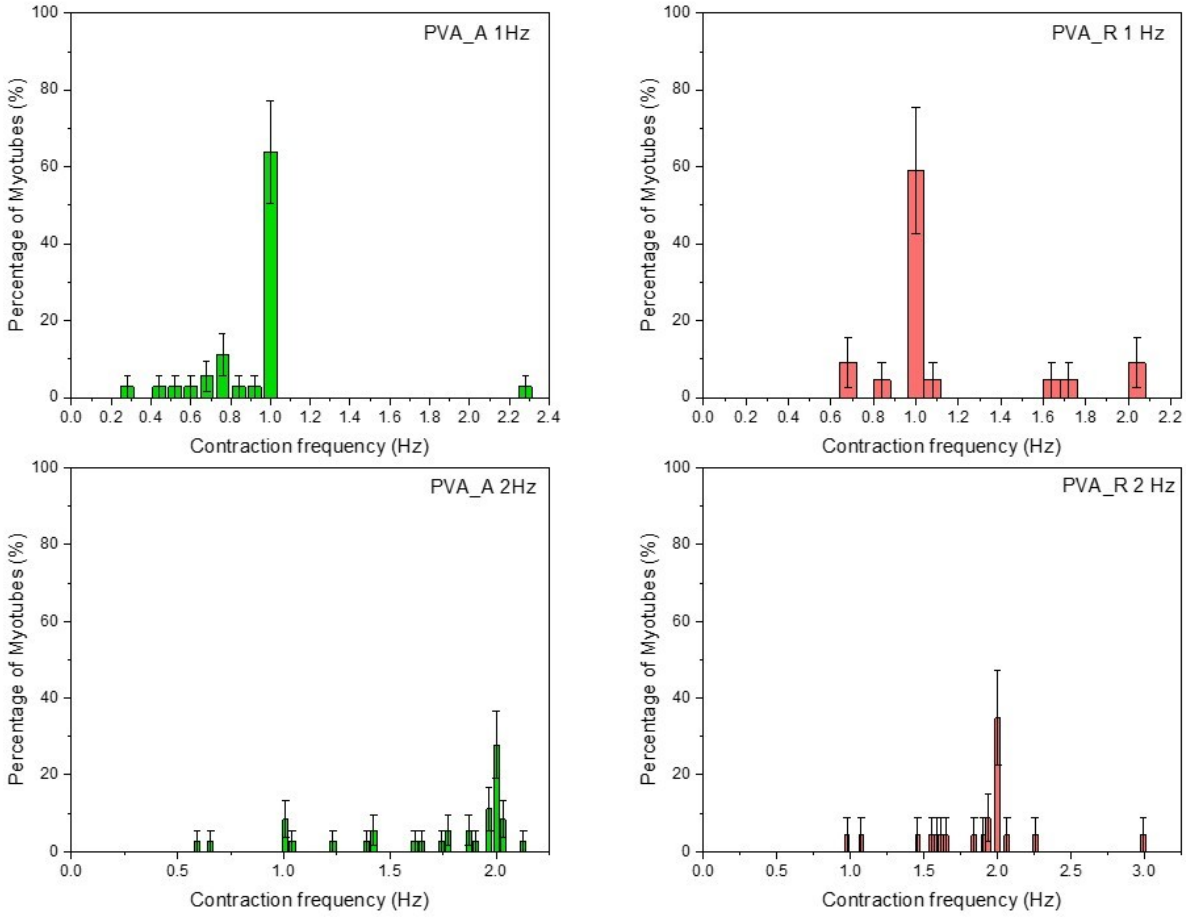


*Figure S5. Histograms represent the mean contraction frequency of myotubes photostimulated at 1 and 2 Hz in aligned and random fibers of PVA. A non-parametric Student's one way ANOVA was performed to evaluate differences between myotubes stimulated at different frequencies within each sample type. The results showed significant differences in PVA aligned and random samples for each stimulation frequency (aligned **p= 0.001, random * p=0.04).*

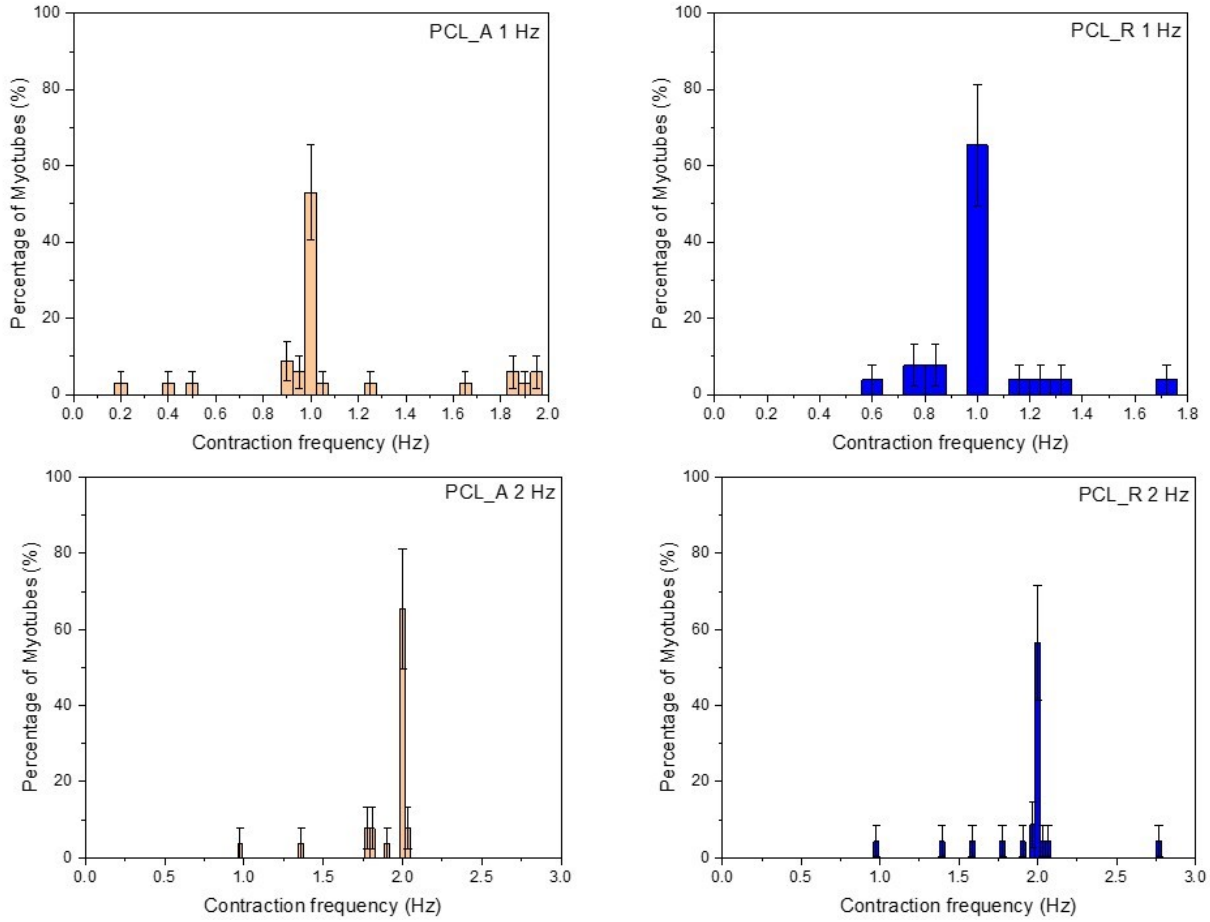


*Figure S6. Histograms representative of the mean contraction frequency of myotubes photostimulated at 1 and 2 Hz in aligned and random fibers of PCL. A non-parametric one-way ANOVA was performed to evaluate differences between myotubes stimulated at different frequencies within each sample type. The results showed significant differences in PCL aligned and random samples for each stimulation frequency (aligned **p= 0.0046, random * p=0.049).*

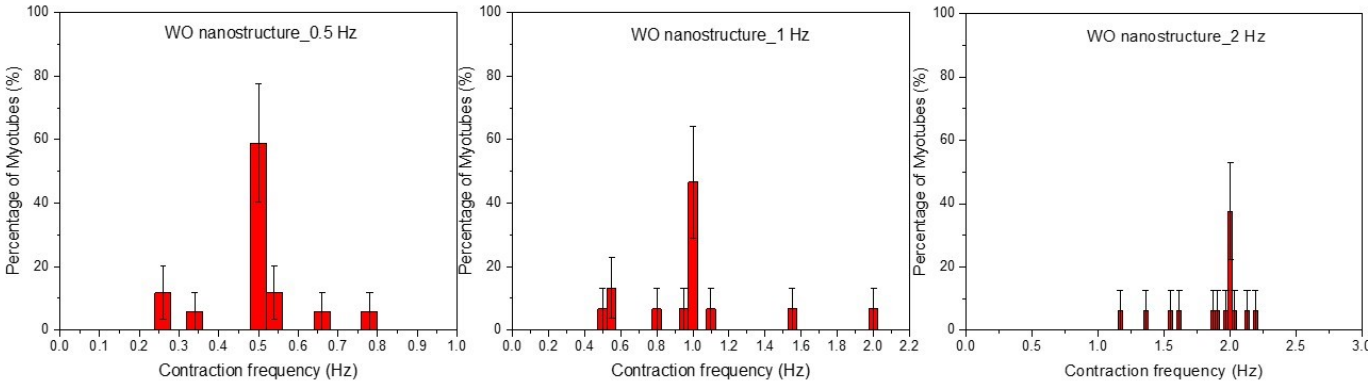


Figure S7. Histograms representative of the mean contraction frequency of myotubes photostimulated at 0.5, 1 and 2 Hz 2D planar surface without any nanostructure.

Confocal Imaging

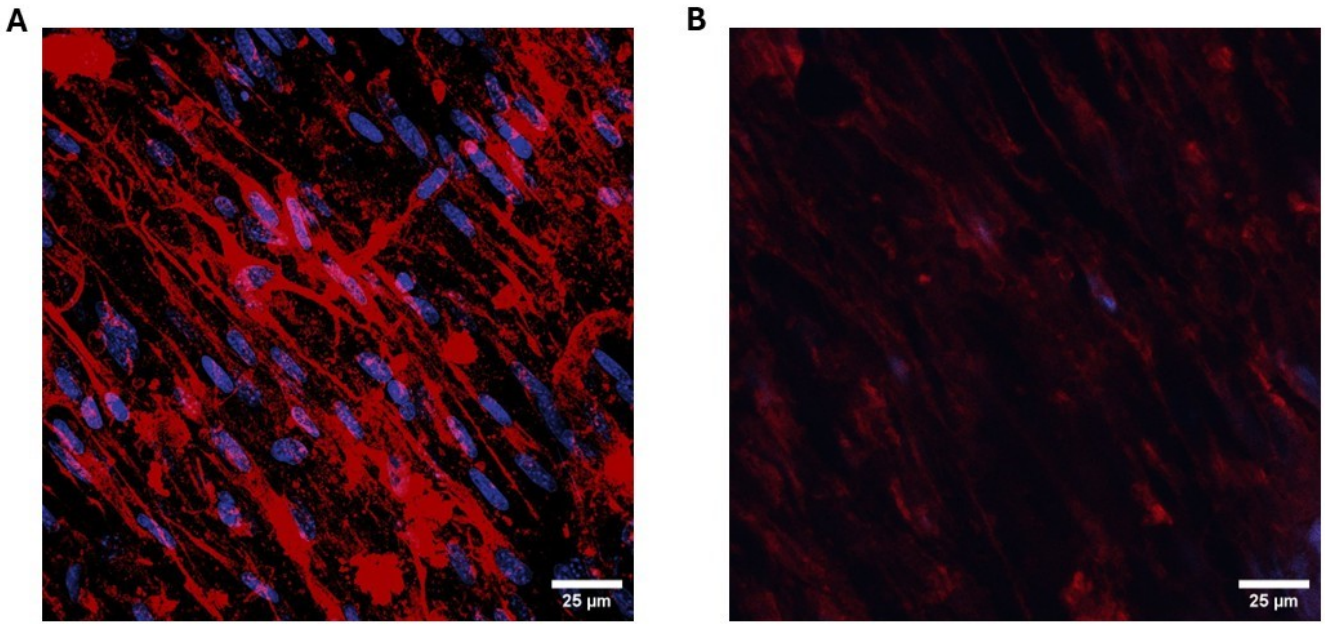
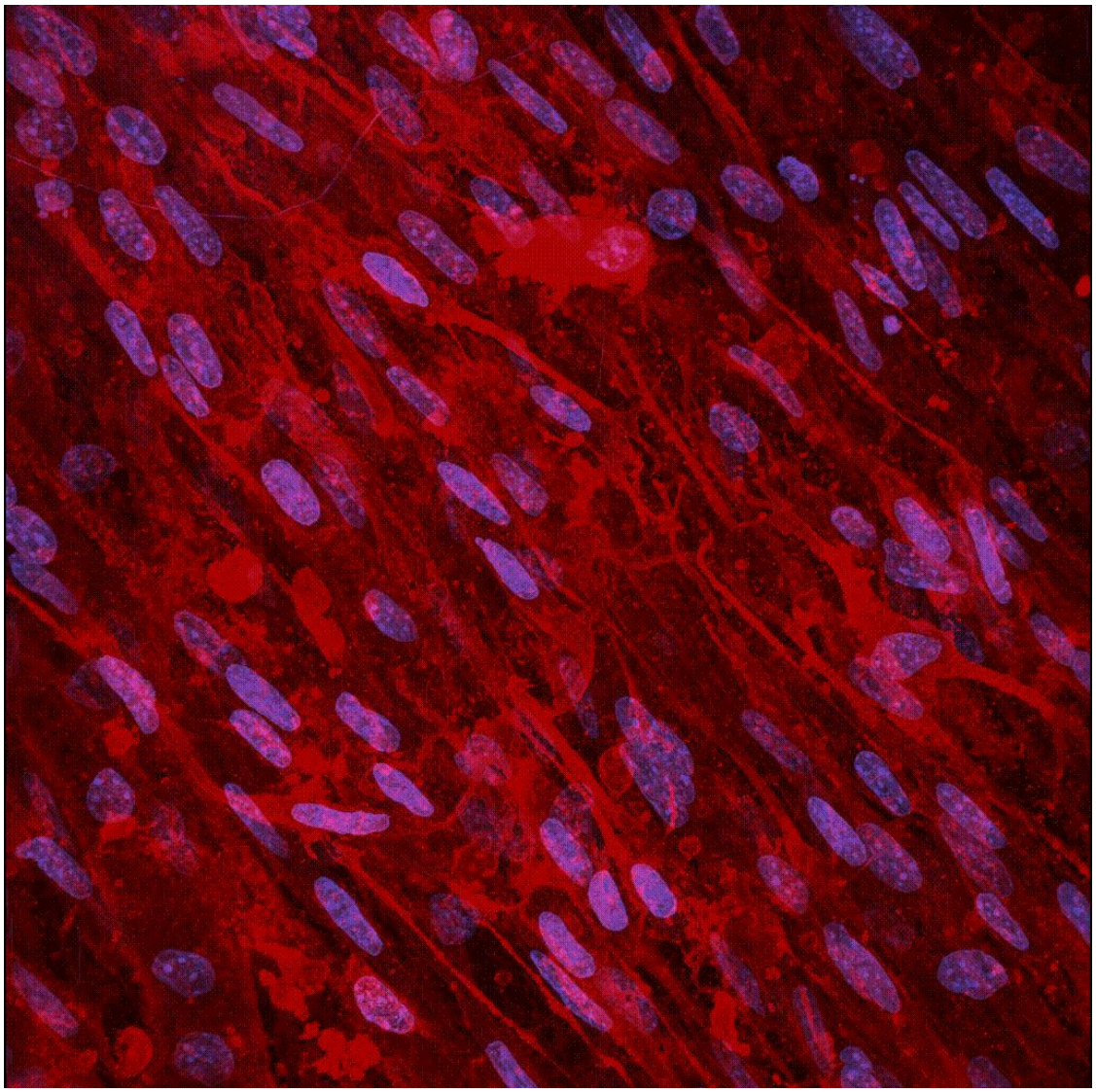


Figure S8. Confocal images of differentiated C2C12 on self-standing PVA membranes A) Maximum intensity projection, B) Minimum intensity projection.



Video 1. 3D rendering of confocal z-stack slices of C2C12 myotubes on a PVA self-standing membrane.

Mechanical characterization

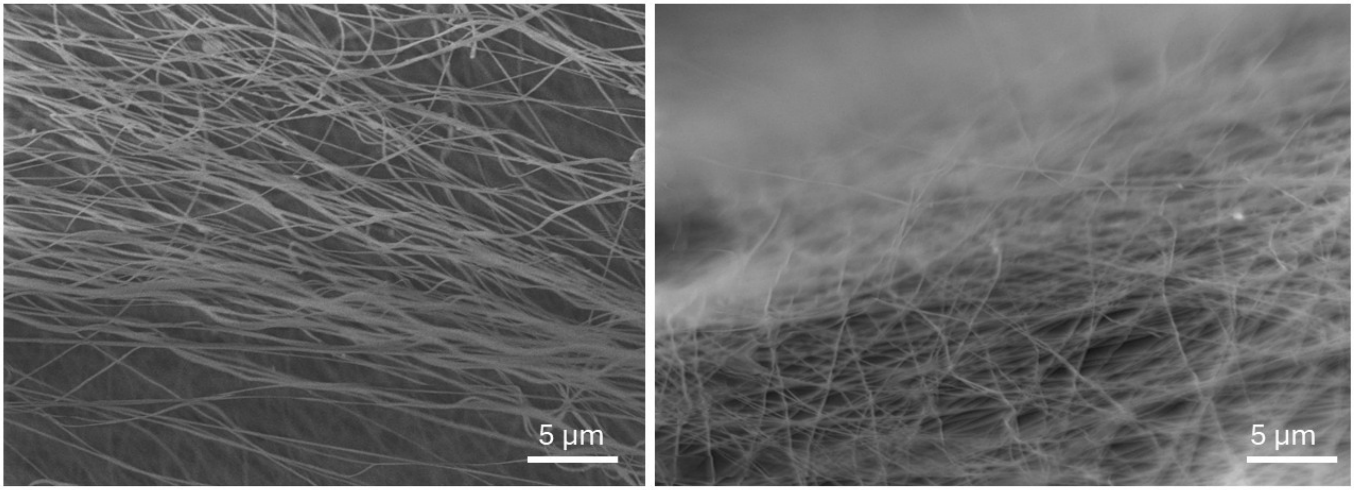


Figure S9. SEM images of PVA aligned ES membranes.

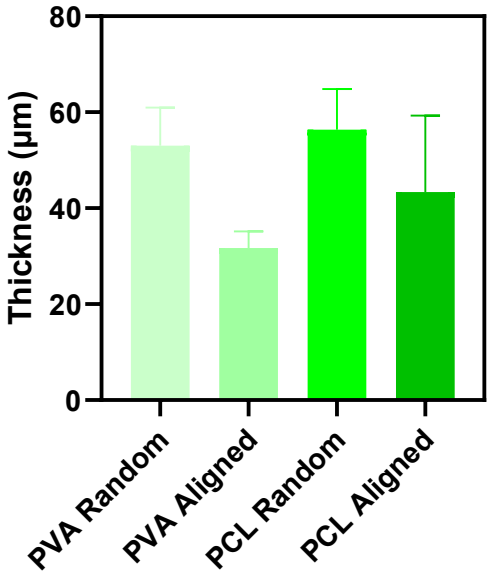


Figure S10. Electrospun membranes thickness for PVA and PCL in aligned and random fibers orientation. A t-student was performed for statistical analysis.

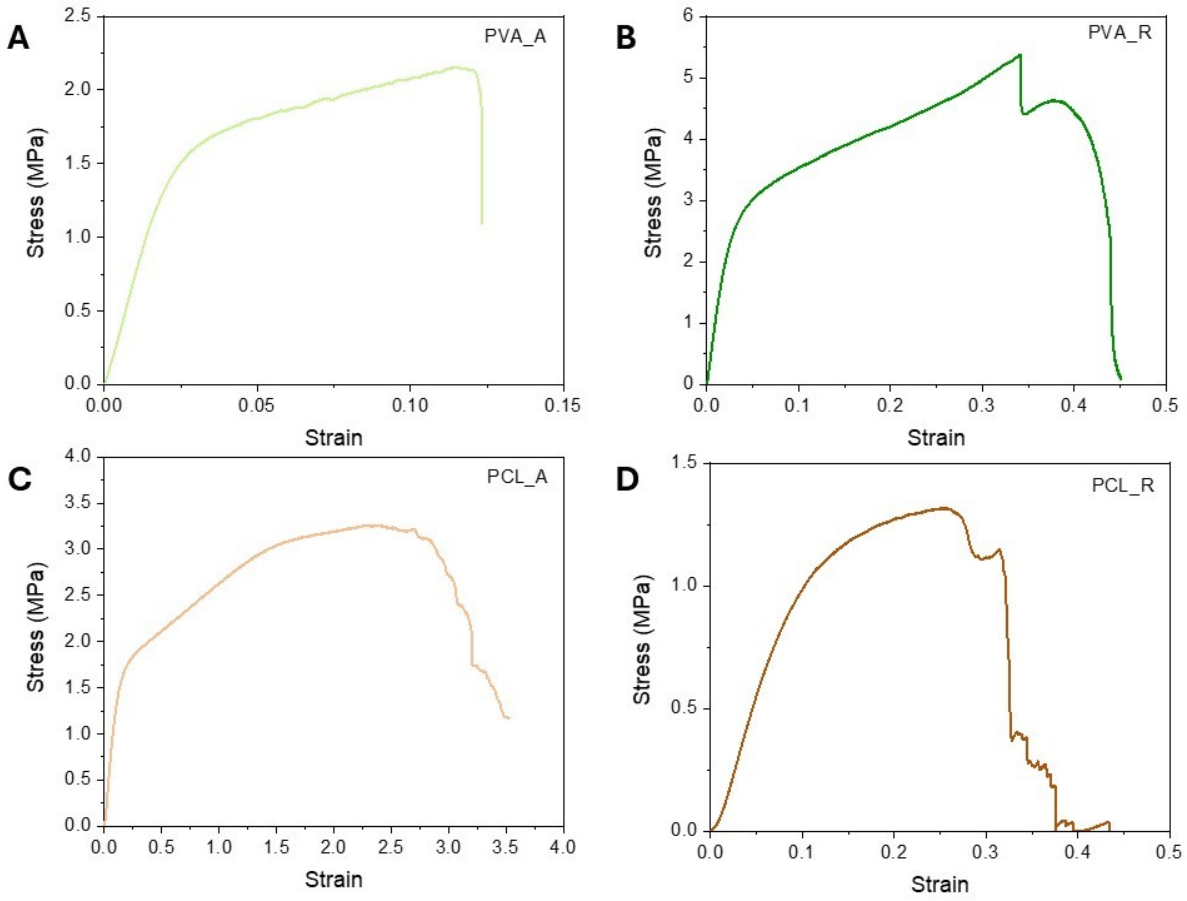


Figure S11. Representative stress-strain curve, from which Young's moduli were obtained by fitting the linear elastic region for A) PVA aligned, B) PVA random, C)PCL aligned and D) PCL random membranes.

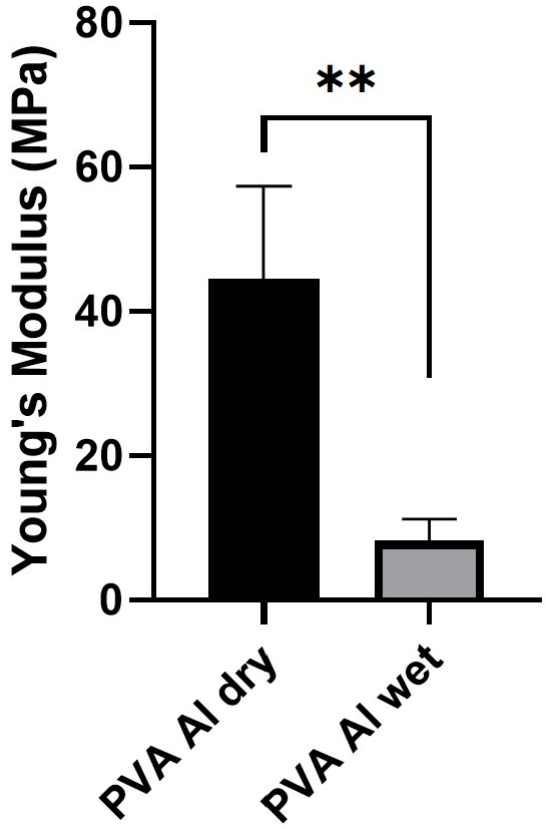
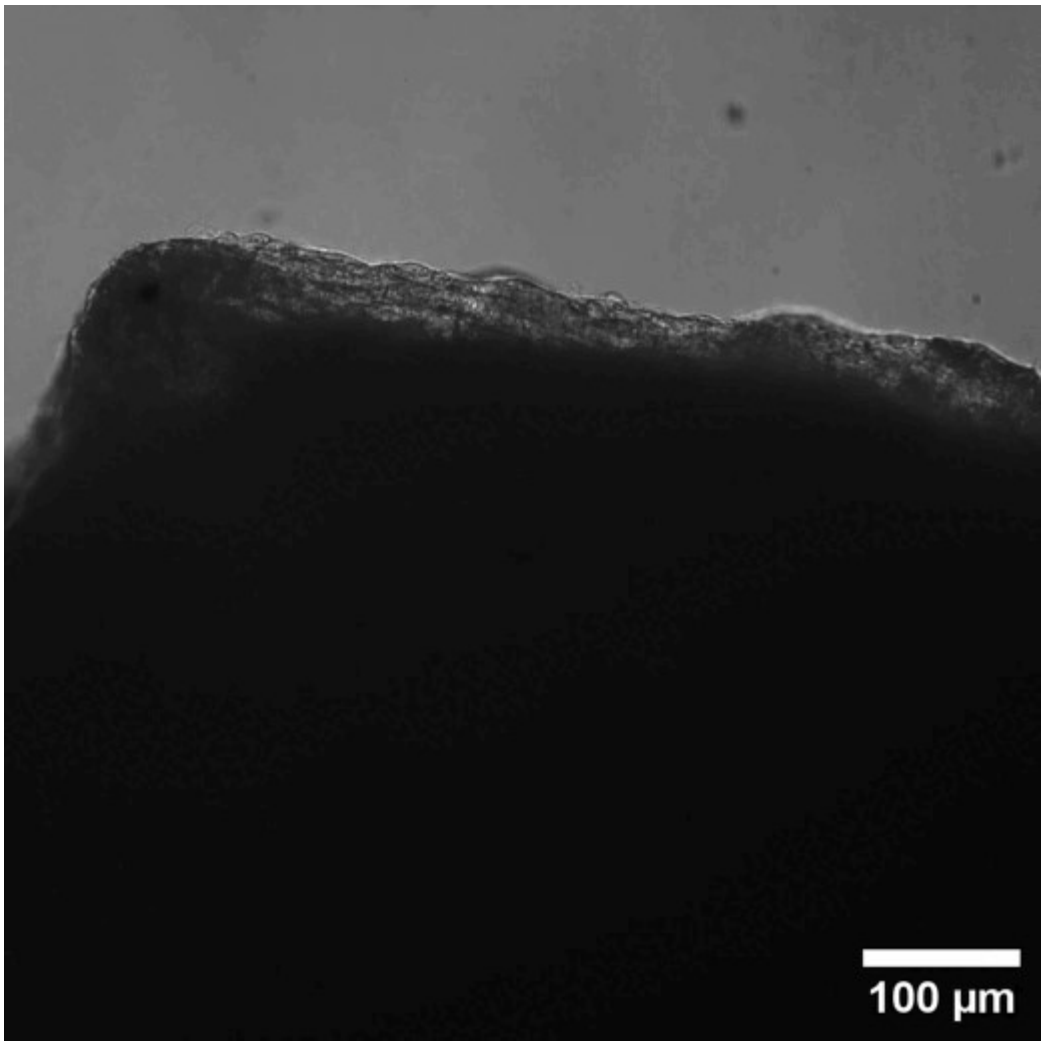
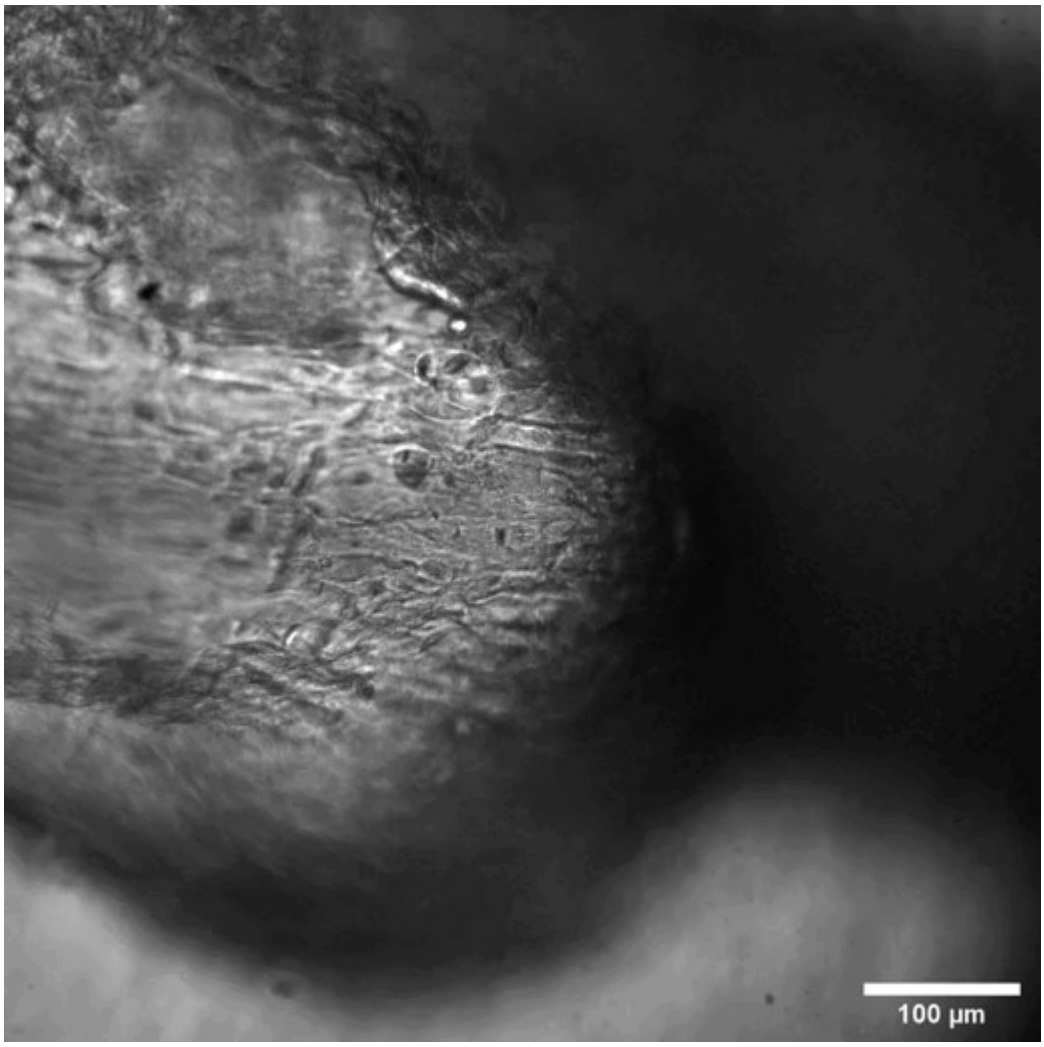


Figure S12. Decrease in the Young's modulus of the PVA electrospun membrane after 24 hours of incubation in cell medium.



Video 2. Video obtained from optical microscope during electrical stimulation of PVA Aligned membrane at 1 Hz using an objective 20x.



Video 3. Video obtained from optical microscope during photostimulation of PVA Aligned membrane at 1 Hz using an objective 20x.

Biomechanical analysis under electrical stimulation

Electrical stimulation induced an average ES-membrane displacement (ΔL_e) of 4.9 µm, while the best performing sample yielded a displacement of 7.7 µm. In terms of generated force, electrical stimulation permits to reach 890 µN with a corresponding stress (σ) of 6.3 kPa. Using the average active tension generated by a single myotube (F_{sm} = 0.88 µN)[42] we performed an approximate estimation of the number of myotubes required to generate 890 µN of force which corresponds to approximately N=10^3 contractile myotubes. Assuming the dimensions used for the ES membranes, the available volume for cell growth and differentiation is about 3.5 mm^3. Considering porosity, the "available space" V is about 2 mm^3. Based on an average myotube size with a diameter of 20 µm and length of 300 µm, the estimated volume occupied by a single myotube is approximately v=10^{-4} mm^3 and the total myotube volume is (N x v) = 10^{-1} mm^3. This means that myotubes fill up only about 5% of the available space.

Sample	Thickness	Length	Width	ΔL_e	E	A_0	A_{ef}	σ_e
PVA aligned	35±3 µm	10 mm	10 mm	4.9±1 µm	8.26 MPa	0.35 mm^2	0.14 mm^2	4.1±1.5 kPa

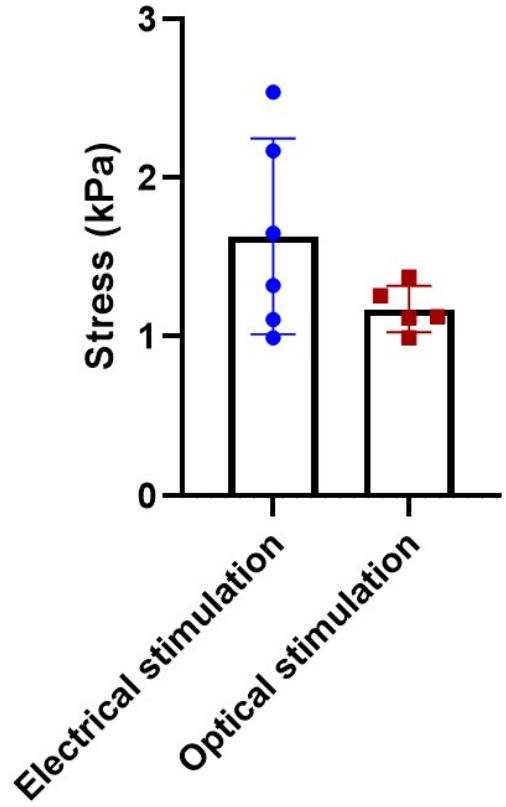


Figure S13. Stress generated in PVA-aligned electrospun membranes during electrical and optical stimulation at 1 Hz, after 12 days of myotube differentiation.