Electronic Supplementary Material (ESI) for RSC Advances.

Electrospun Acellular Scaffolds for Mimicking the Natural Anisotropy of Extracellular Matrix

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Supplementary Information

Fabrication of PCL and PCL-PVDF scaffolds

A solution of PCL (Mw= 70,000 GPC; Scientific Polymer Products, USA) was prepared by dissolving 20% PCL in a ratio 3:2 of chloroform (Sigma Aldrich, USA) and methanol (Sigma Aldrich, USA). The solution was electrospun at 12kV with a fluid flow rate of 1.5ml/h and rotating collector speed of 400 rpm. PCL-PVDF fibers was prepared by electrospinning from two syringes, 20% PCL in chloroform (fluid flow: 0.24ml/h), and a solution of PVDF (Mw= 534,000; Sigma Aldrich; France) prepared by dissolving 20% PVDF (fluid flow: 0.12ml/h) in dimethylformamide (Macron Fine Chemicals; USA) and acetone (Sigma Aldrich, USA) in the ratio 7:3 respectively. The fibers were collected on a rotating collector at 2000 rpm by maintaining a potential difference of 20kV. The distance from the nozzle to the collector was maintained at 0.17m.

Characterization of PCL and PCL-PVDF scaffolds

FESEM

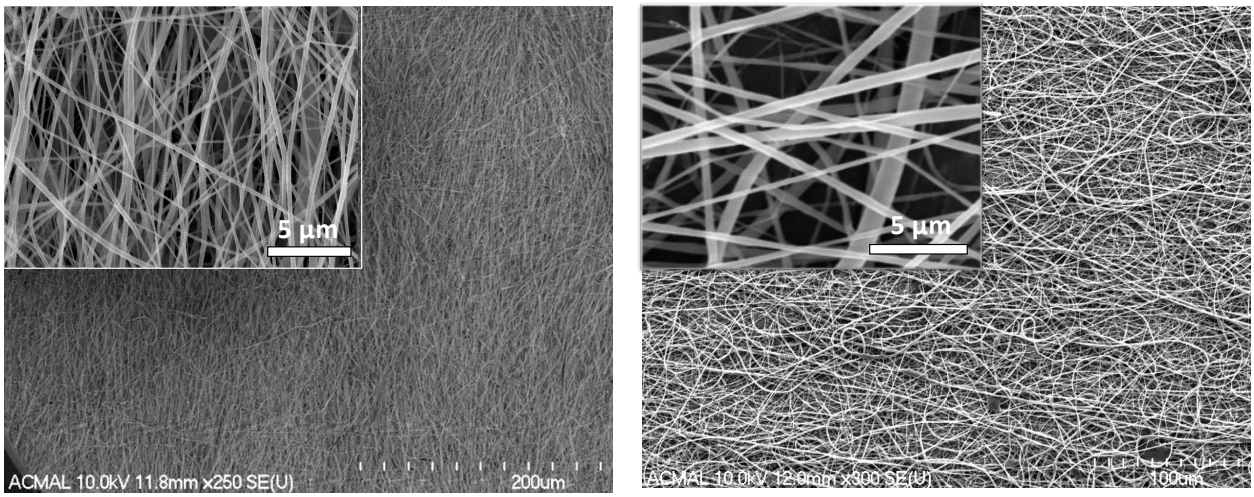

Figure S1. Field emission scanning electron microscopic (FESEM) images of the PCL (left) and PCL-PVDF (right) scaffolds exhibiting aligned morphology and defect free fibers. The inset shows a high magnification image of the fibers. Defect-free nanofibers were obtained using electrospinning. The scaffolds have a high orientation of fibers in a single direction and different layers forming a 3D mat.

ATR-FTIR

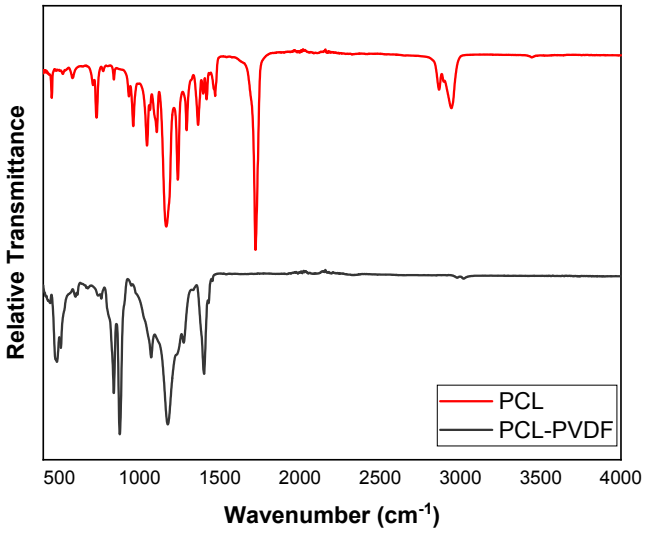

Figure S2. Fourier transform Infrared Spectroscopy (FTIR) spectra of the PCL and PCL-PVDF scaffolds. The significant peaks corresponding to PCL and PCL-PVDF were identified on the spectrum. Peaks corresponding to the β-state of PVDF was identified on the PCL-PVDF scaffold.

Infrared spectroscopy was used for analyzing the surface functional groups of the three samples. As shown in figure S2, the peaks at 1722 cm^{-1} (C=O stretching), 1166 cm^{-1} (C-O stretching), 2944 cm^{-1} and 2866 cm^{-1} (symmetric and asymmetric CH_2 stretching respectively) are unique to PCL. The high intensity peak at 1293 cm^{-1} corresponds to the C-O and C-C stretching in the crystalline phase of PCL. The PCL-PVDF scaffolds had peaks at 840 cm^{-1} (CH_2 rocking) can be attributed to both the γ-phase as well as the β-phase of PVDF. Further peaks at 1275 cm^{-1} (C-F out of plane deformation) and 510 cm^{-1} (CF_2 bending) corresponds specifically to the β-phase of PVDF. The characteristic peaks of α-phase and γ phase of PVDF were also present at 762 cm^{-1} (CF_2 bending and scelete bending) and 781 cm^{-1} (CH_2 rocking). The distinctive peaks of PCL were also present in the PCL-PVDF scaffolds. The β-phase corresponding to PVDF in PCL-PVDF scaffold is at a higher concentration in relation to the other electroactive forms of PVDF. This can be attributed to the high electric field during electrospinning while fabrication of scaffolds which annuls the need for post-processing by stretching or electric poling.[1, 2]

XRD

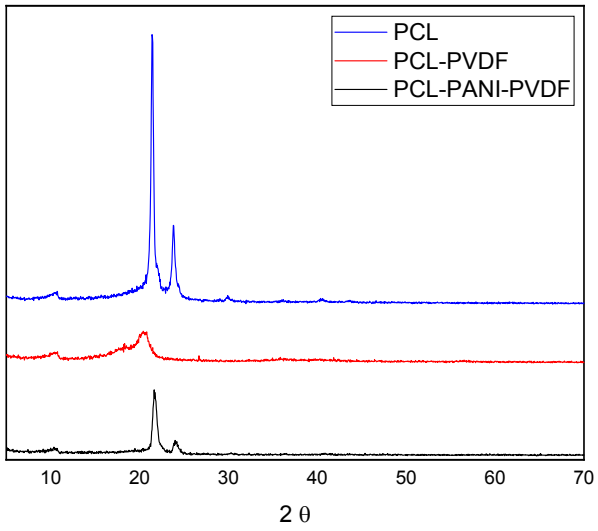

Figure S3. X-Ray Diffraction (XRD) spectra of the PCL, PCL-PVDF and PCL-PANI-PVDF scaffolds plotted by relative Y-offset. The spectra corresponding to PCL scaffold (blue) has higher intensity of peaks (high crystallinity) from PCL while the PCL-PVDF (red) has reduced intensity of prominent peaks (low crystallinity) from PCL. The peaks corresponding to PCL is prominently present in the PCL-PANI-PVDF scaffolds.

The XRD analysis of the three polymers is shown in fig. S3. The narrow peaks at 21.30 and 23.70 correspond to the crystalline nature of PCL formed by C-O and C-C stretching and the d-spacing of these peaks were calculated to be 4.15 Å and 3.73 Å respectively.[3] The degree of crystallinity of PCL was calculated to be 0.6452. The peaks were indexed to (110) and (200) lattice planes of the orthorhombic unit cell. The spectra from PCL-PVDF showed no major peaks and the degree of crystallinity was calculated to be 0.21%. The presence of peaks corresponding to PANI is unclear because of the high signal to noise ratio in the low angle region and low intensity reads of the polymer sample.

DMA

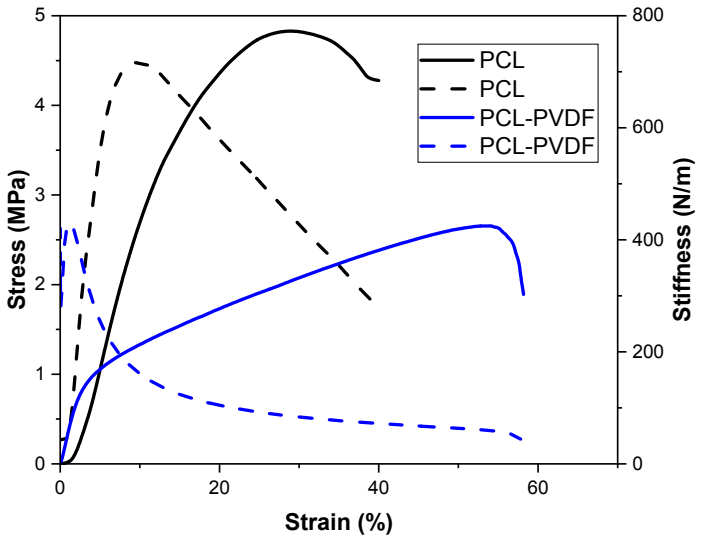

Figure S4. The mechanical characterization of the scaffolds using Dynamic mechanical analyzer was done in similar conditions as PCL-PANI-PVDF. The dotted lines correspond to the Stiffness-strain while the solid lines correspond to the stress-strain profile of the PCL and PCL-PVDF scaffolds.

Cell Characterization of scaffolds

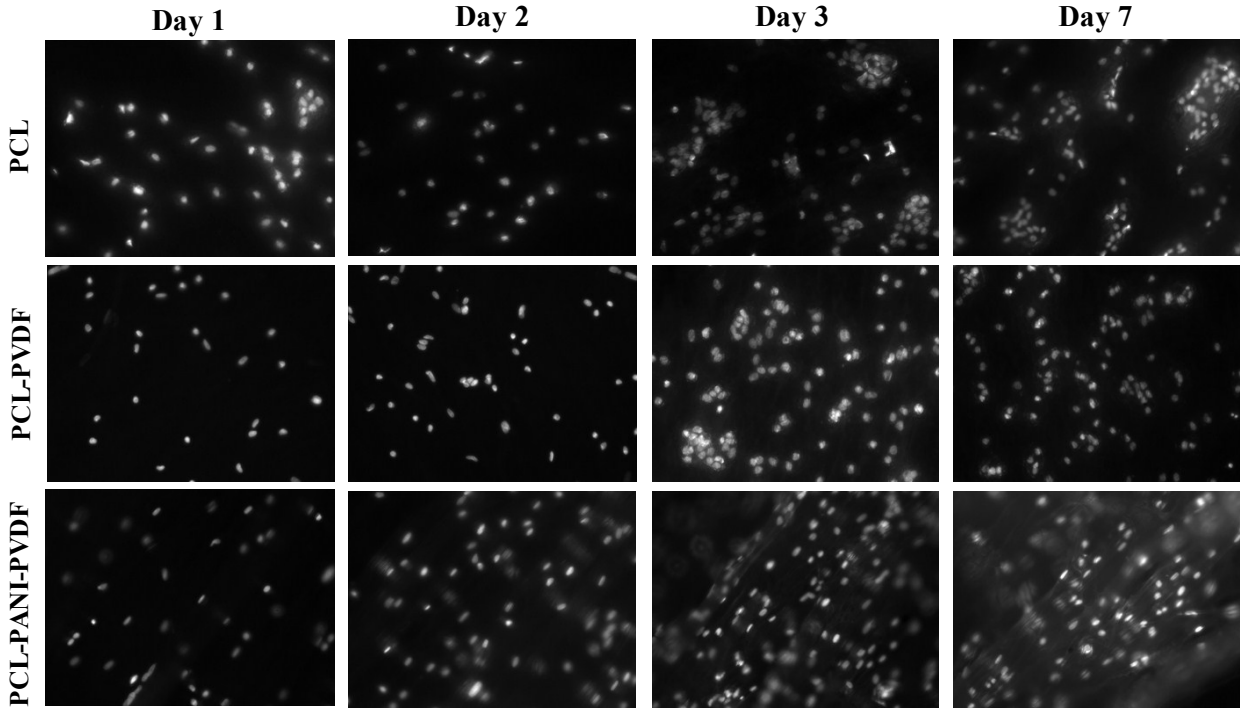

Figure S5. Fluorescent microscope images of rat cardiomyoblasts (H9c2) cells on PCL, PCL-PVDF and PCL-PANI-PVDF on days 1, 2, 3 and 7. The nuclei were stained with DAPI (white). The shape of the nucleus is clearly visible. The cells start clustering in PCL scaffolds as inferred from the images on Day 3 and Day 7. The cells are less clustered in PCL-PVDF as compared to PCL, however there is still some clumping present. The cells are spread out completely in the PCL-PANI-PVDF scaffold and are aligned in the same orientation. Images are at 20X magnification.

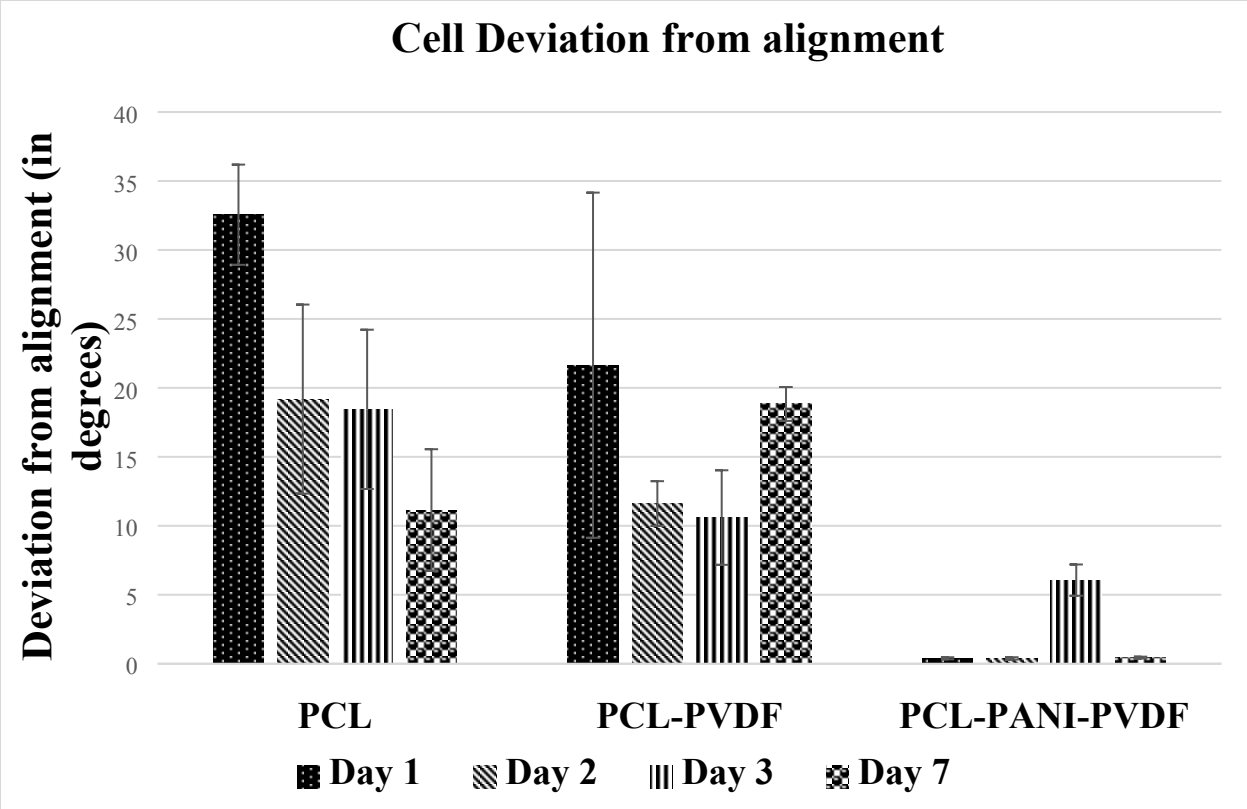

Figure S6. The cell deviation from orientation on each scaffold was analyzed using the fluorescent images. PCL-PANI-PVDF had the best cellular alignment as compared to the other two scaffolds. The data was represented as mean± standard error of mean from n=5.

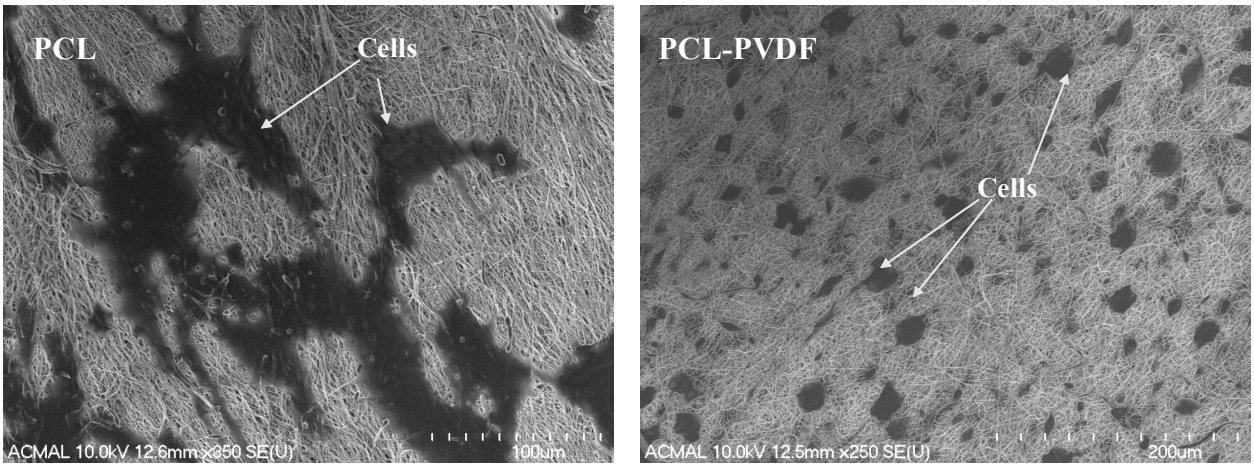

Figure S7. Field Emission Scanning electron microscopy (FESEM) images of rat cardiomyoblasts (H9c2) cells on PCL (left) and PCL-PVDF (right) on day 3 after cell seeding.

References

1. T. Lei, L. Yu, G. Zheng, L. Wang, D. Wu and D. Sun, *J. Mater. Sci.*, 2015, **50**, 4342-4347.
2. A. Baji, Y.-W. Mai, Q. Li and Y. Liu, *Nanoscale*, 2011, **3**, 3068-3071.
3. X. Wang, H. Zhao, L.-S. Turng and Q. Li, *Industrial & Engineering Chemistry Research*, 2013, **52**, 4939-4949.