ELECTRONIC SUPPLEMENTARY INFORMATION (ESI)

Permanently Hydrophilic, Piezoelectric PVDF Nanofibrous Scaffolds Promoting Unaided Electromechanical Stimulation on Osteoblasts

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Supplementary Figures

1. Drop-cast scaffolds structure

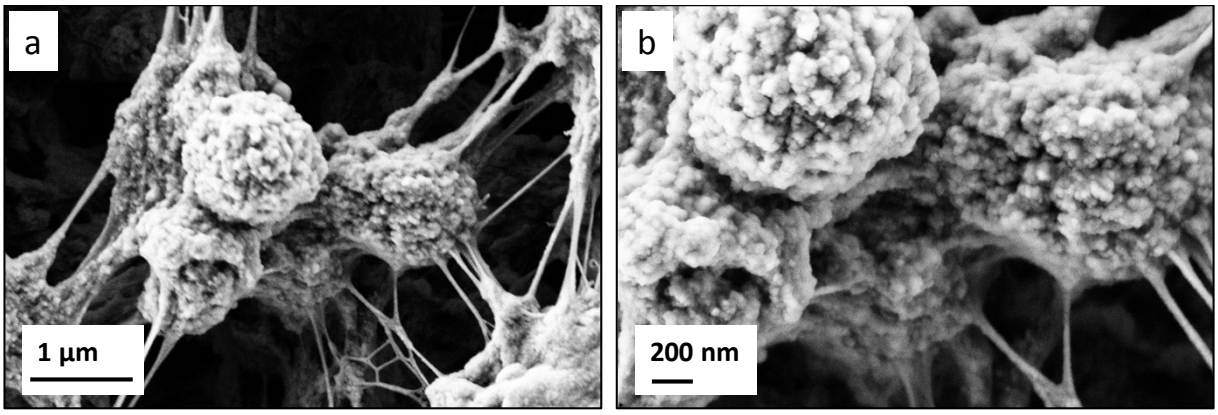

Fig. S1 SEM images of drop-cast scaffolds at different magnifications. The rough surface of the particles is composed of nano-grains with a diameter typically in the range of 60-100nm.

2. Hydrophilicity stability of plasma-treated PVDF scaffolds

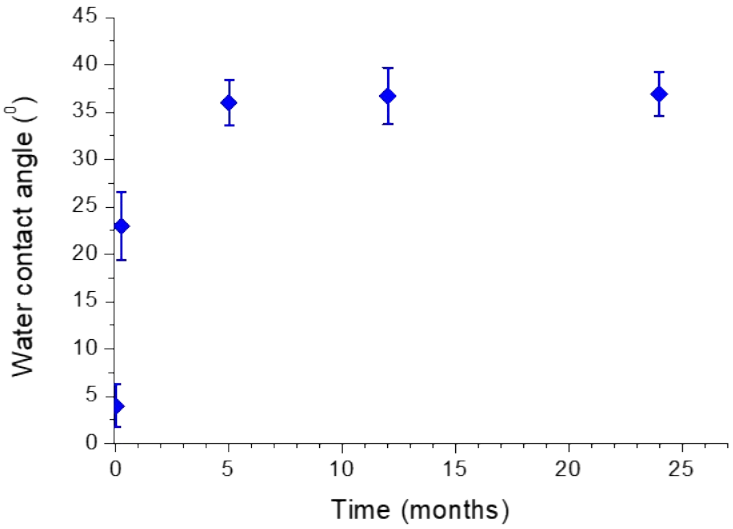

Fig. S2 Water contact angles of electrospun scaffolds after oxygen plasma treatment for a period of 2 years.

3. Topography effect on PVDF surface due to plasma treatment

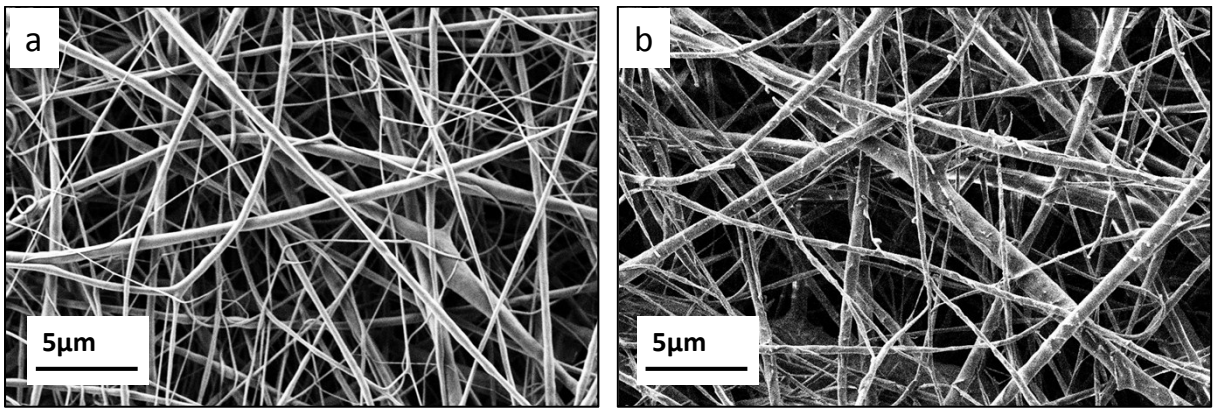

Fig. S3 SEM images of electrospun and drop-cast scaffolds (a) before and (b) after treatment with oxygen plasma. The mean fiber diameter of the electrospun scaffolds is 377 ± 234nm. Oxygen plasma treatment modified not only the surface chemistry but also the topography, by slightly etching the surface of the scaffolds. Nevertheless, this etching did not alter fibers' thickness significantly and subsequently scaffolds porosity.

4. XPS analysis

XPS data were also recorded in order to confirm the cleanliness of the pristine PVDF scaffolds and to assess the degree of defluorination after plasma treatment. Fig. S4a present the survey spectrum of the pristine PVDF showing the presence of fluorine (F1s) and carbon (C1s) as the main components of the material, with no oxygen observable there. High resolution spectra for the C1s and O1s regions are presented on Fig. 2; the O1s spectrum confirms the quasi absence of oxygen on the membrane (0,2 %, see Table 2), while the C1s presents 2 main features at 286.6 eV (C_1) and 291.2 eV (C_2) assigned, respectively to carbon atom in -**C**H_2-CF_2- and -CH_2-**C**F_2- environments. One can also note the presence of a small feature at low binding energy, C_3 at 285.0 eV, assigned to aliphatic residual carbon contamination upon XPS analysis. When looking at the atomic percentage weight of the C_1 and C_2 component (Table 1), the experimental ration is 0.99 (26.65:26.8) when the theoretical one should be 1, confirming that way the good stoichiometry of the (CH_2-CF_2) polymers motives in the PVDF scaffolds. In addition, Table 2 presents the different atomic ratio obtained from the XPS data, and for the electrospun PVDF

scaffold, the value of F/C is equal to 0.85, which is consistent with a clean PVDF membrane.[1] The small difference observed with the theoretical ratio of 1 could be attributed to the presence of adventurous carbon (from contamination) and also from the fact that this ratio is the theoretical one from the raw bulk material, while the XPS analysis provides more an extreme surface analysis with all interfacial effects associated to it. Nevertheless, our data confirm the very clean chemical composition of pristine PVDF scaffolds, with a negligible amount of oxygen, unlike most of the data reported in the literature showing an excess of oxygen or contamination with carbon.[2–4]

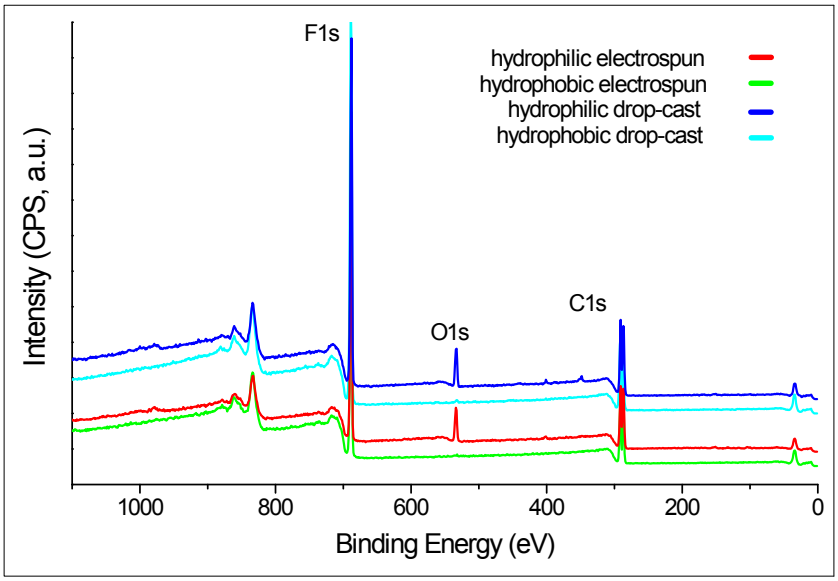

Fig. S4 Survey X-Ray Photoemission spectra of electrospun (a, b) and drop-cast (c, d) PVDF before and after plasma treatment.

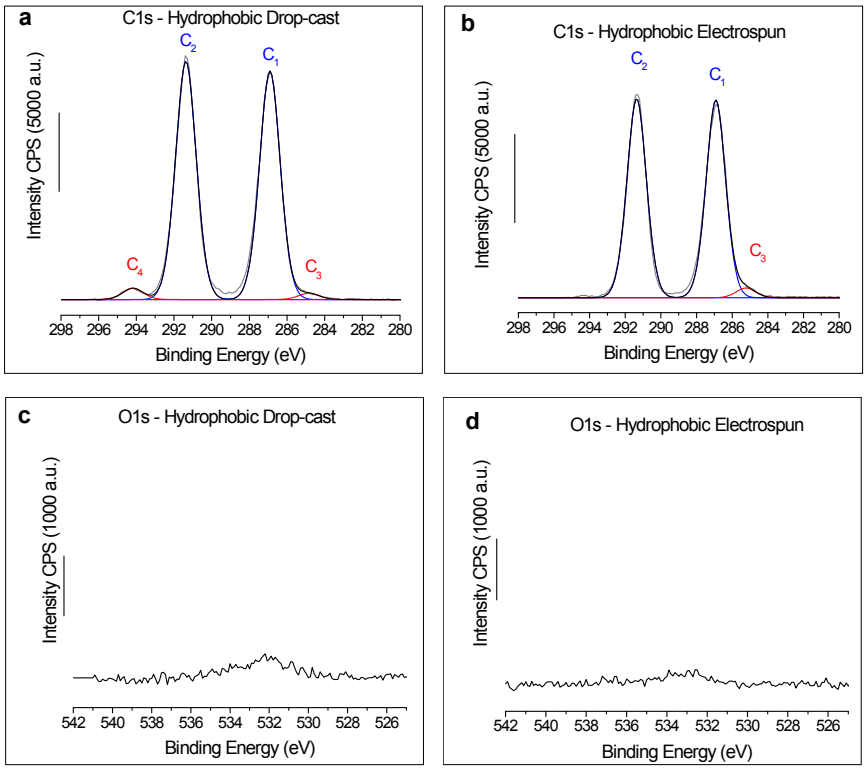

Fig. S5 C1s and O1s High Resolution XPS Spectra of PVDF (a) and (c) drop-cast and (b) and (d) electrospun.

5. Electromechanical measurement of the electrospun scaffold

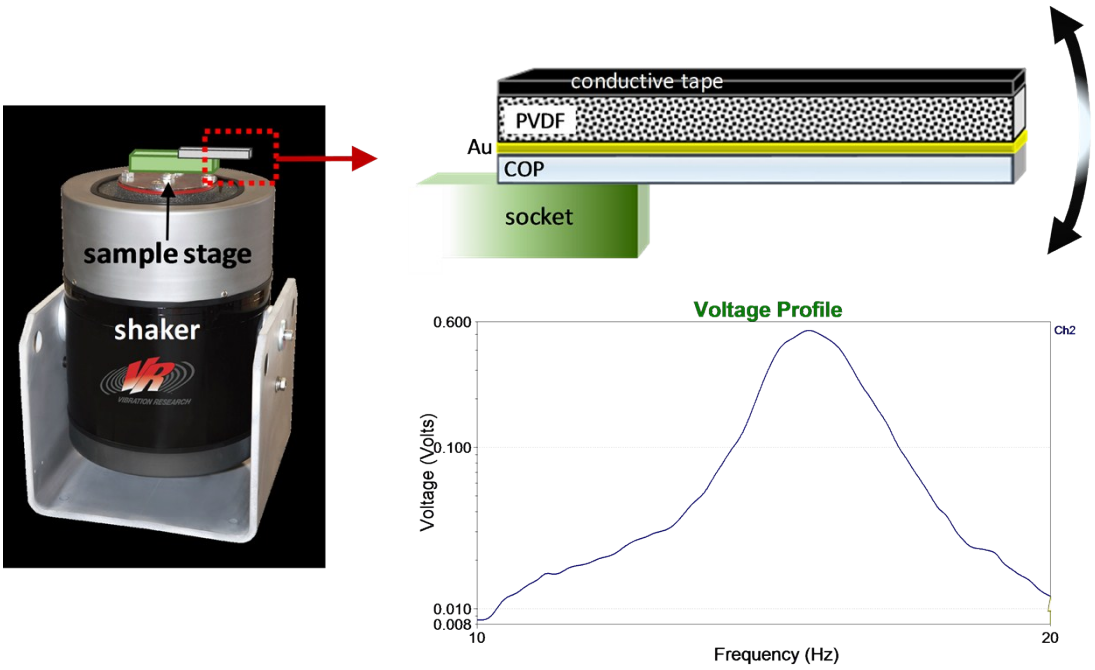

Fig. S6 Electromechanical testing: Scheme of the flexible test device and its frequency response.

6. Viability of Saos-2 cells

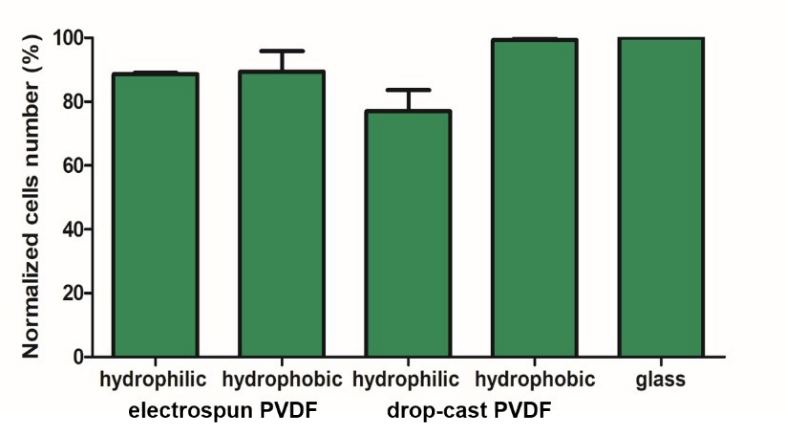

Fig. S7 Normalized number of cells grown on all samples after 3 days in culture. These values derived by dividing the total number of cells of each scaffold with those on glass control.

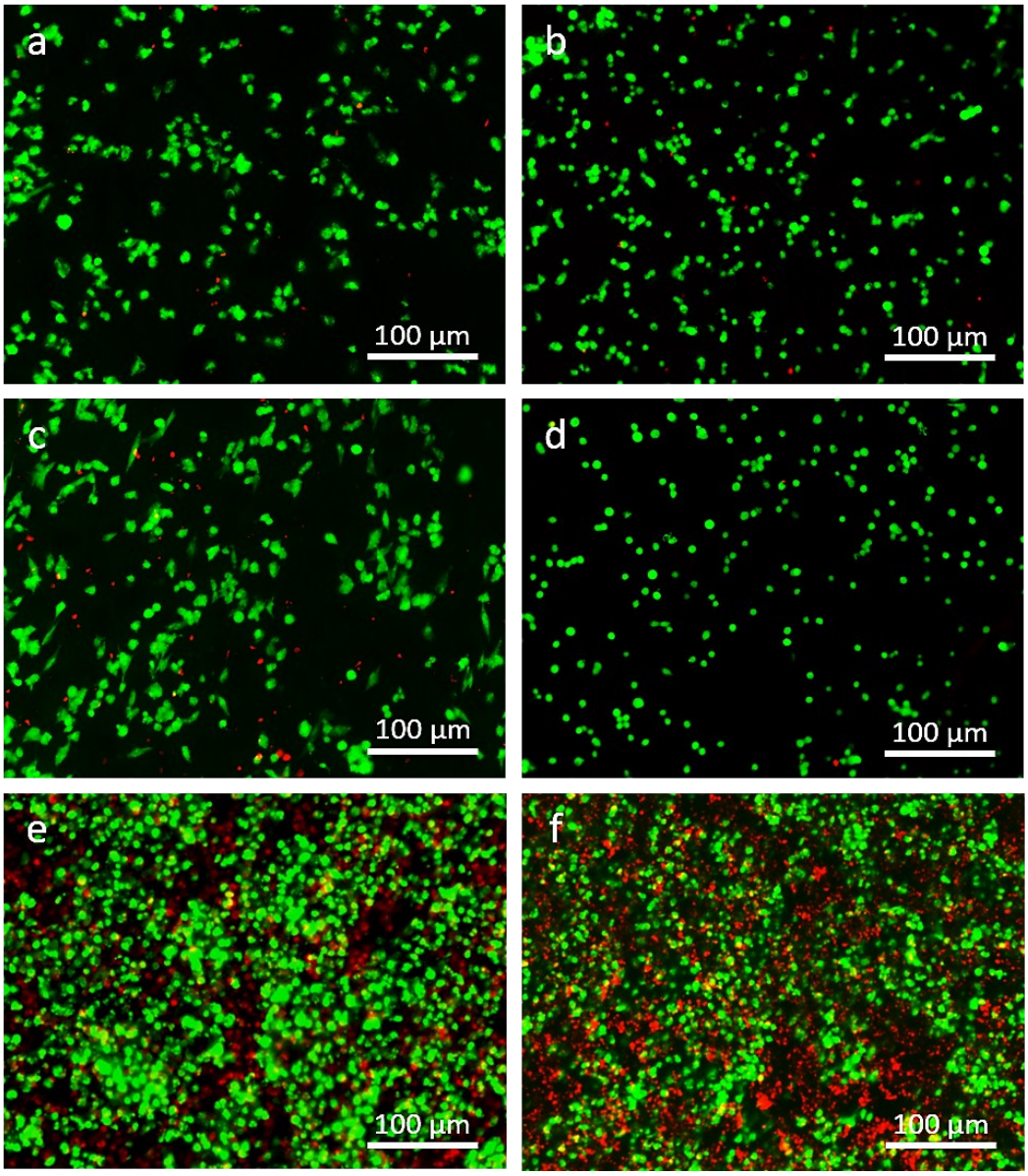

Fig. S8 Viability of Saos-2 cells cultured onto hydrophilic (a) and hydrophobic (b) PVDF drop-cast, and hydrophilic (c) and hydrophobic (d) PVDF electrospun scaffolds for 3 days. Cells cultured onto hydrophilic (e) and hydrophobic (f) electrospun PVDF for 30 days. Live cells are stained in green whereas dead cells are stained in red.

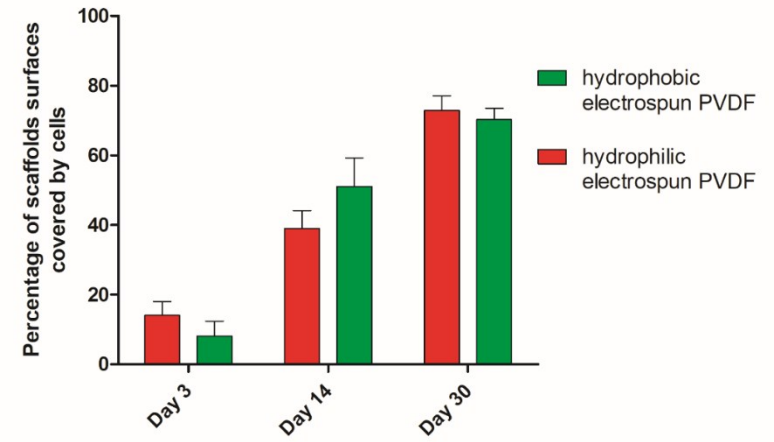

Fig. S9 Percentage of electrospun scaffolds surfaces covered by cells at 3 different time points: 3, 14, and 30 days after culture on the hydrophilic and hydrophobic electrospun PVDF.

7. Simulation of the generated piezopotential by a single PVDF nanofiber

The simulation software COMSOL 5.1, was used to calculate the piezopotential generated by a single PVDF nanofiber when strained with a magnitude similar to this produced by a cell. In specific, the Piezoelectric Module of COMSOL was applied, where both extremes of a PVDF nanofiber were anchored and grounded and a force ranging from 0.1 nN to 10 nN was loaded in the middle of the piezoelectric fiber. The results show that for a length of 10 μm and a radius ranging from 50 nm to 200 nm, a maximum piezopotential of around 30 mV can be obtained.

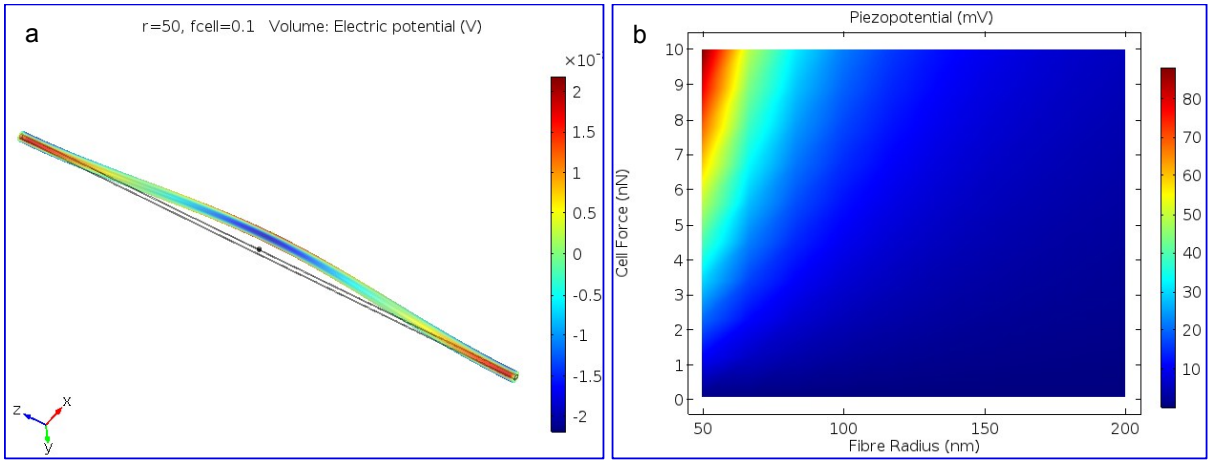

Fig. S10. Simulation results: (a) a single nanofiber of 100nm diameter, when a force of 0.1nN is applied, (b) piezoelectric potential (absolute value) for different nanofiber diameters and cell forces.

Supplementary Movies

M1. CLSM z-stack of actin fibers (red) in Saos-2 cells cultured on hydrophilic electrospun PVDF

M2. Calcium transients of Saos-2 cells cultured on hydrophilic electrospun PVDF, sped up x100.

M3. Calcium transients of Saos-2 cells cultured on glass control, sped up x100.

References

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