Supporting Information

Pioneering Wound Care Solutions: Triaxial Wet-Spun Fibers with Bioactive Agents for Chronic Wounds – Part II (Controlled release and biological activity of the active agents)

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Section S1. Hollow Fibers Morphology

Physical images of the produced wet-spun hollow fibers were collected, showing the presence of folds and breakable sites (Figure S1).

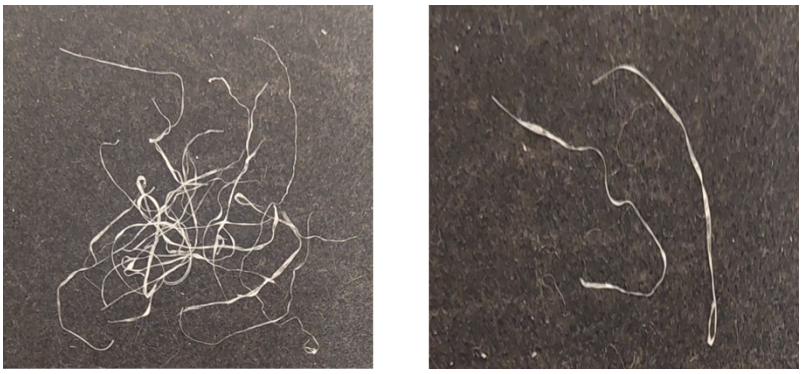

Figure S1. Physical images of SA hollow wet-spun fibers.

Section S2. Chemical characterization of EOs

The chemical composition of CLO was determined via microextraction followed by gas chromatography-mass spectrometry (SPME-GS-MS). The identification of the major component peaks for such EO and retention times were determined (Table S1).

Table S1. Major component peak identification in CLO, determined by gas chromatography (SPME-GS-MS).

Retention time (min)	Compound identification
12.23	eugenol
12.45	2-methoxy-4-(1-propenyl)phenol
13.25	1R,4S,7S,11R-2,2,4,8-tetramethyltricyclo[5.3.1.0(4,11)]undec-8-ene
13.73	5.β-iodomethyl-1.β.-isopropenyl-4.α,5,6,β-bicyclo[4.3.0]nonane

Section S3. Antioxidant Properties of Fibers

A new graph representing the readings at 60 min after different incubation times of fibers in absolute ethanol (1 h, 2 h, 4 h, 6 h and 24 h) is presented below (Figure S2), proving that the incubation times did not significantly influence the DPPH reduction capacities of all samples.

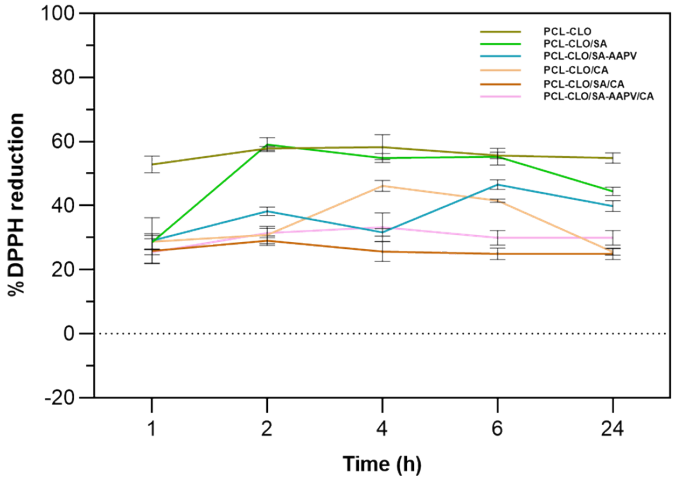

Figure S2. DPPH reduction induced by CLO-loaded wet-spun fibers after 1 – 24 h of incubation. Data are reported as mean ± SD (n = 3).

Section S4. Biofilm Formation

Photographs before washes with PBS were taken, showing the effective formation of biofilms on the wells (Figure S3a). Additional photos were collected at the moment of sample removal from the plate, in which a part of bacterial suspension remained attached to fibrous samples (Figure S3b).

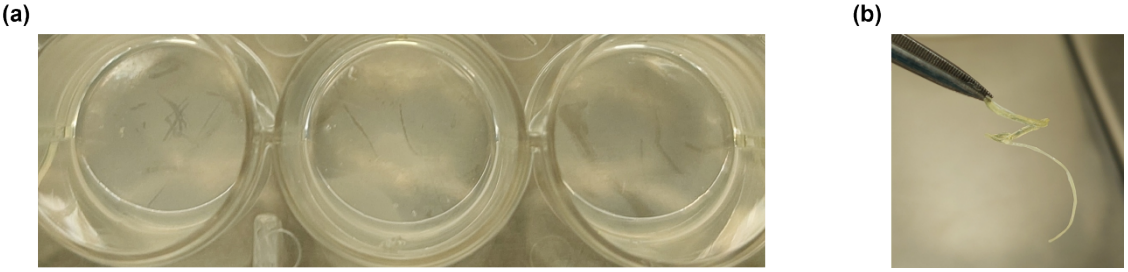

Figure S3. Photographs collected of (a) several plate wells; (b) samples removal from the testing plate.

Section S5. Cytocompatibility Testing

Metabolic activity and cell lysis of all wet-spun fibers was tested for HaCaT and NIH 3T3 cell lines. Prior to any fiber testing, the optimum cell number for each cell line and test was determined for different cell concentration and a positive and negative control, corresponding to 50.000 cells grown in DMEM and DMSO 5% v/v, respectively (Figure S4).

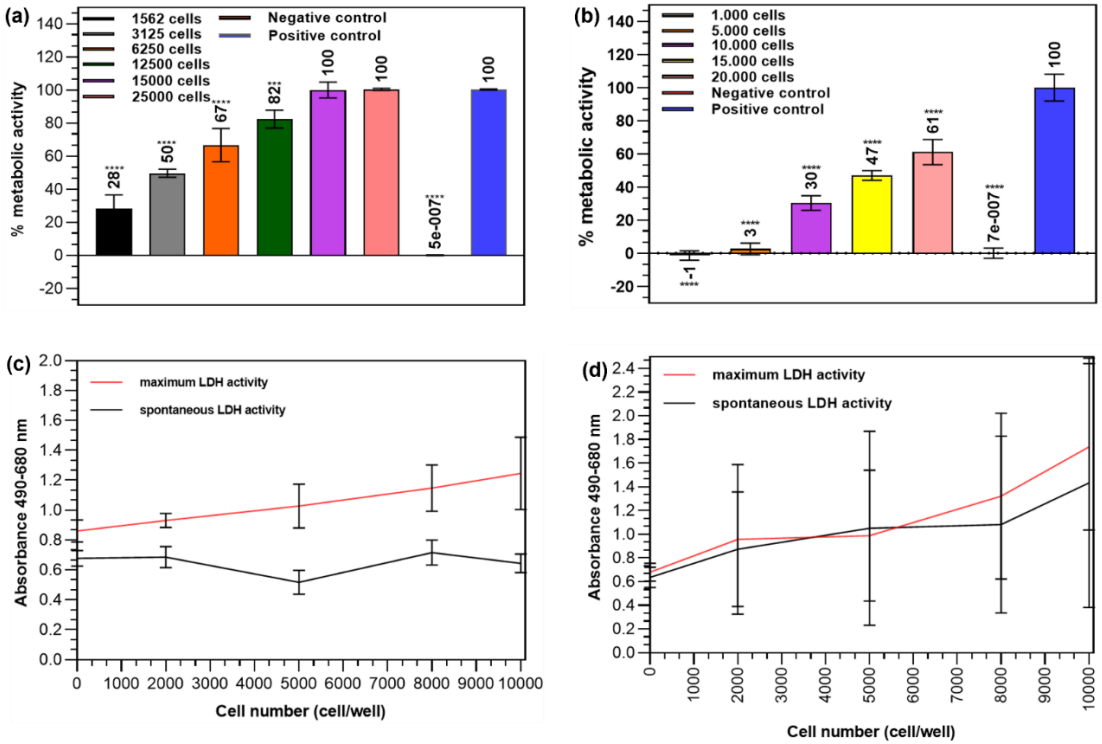

Figure S4. Determination of optimum cell number for alamar blue testing of (a) HaCaT and (b) NIH 3T3; and LDH testing for (c) HaCaT and (c) NIH 3T3 cell lines. Data are reported as mean ± S.D. (n = 4). Statistical significance was determined via Dunnett test applying multiple comparisons between the different cell numbers and each respective positive control (**** $p < 0.0001$; *** $p < 0.004$).

The morphology of HaCaT cell lines was also observed before and after being in contact with all wet-spun fiber typologies for 24 h (Figure S5). Micrographs were acquired with a 10 × magnification objective of 0.25 N.A., using an Axiocam 105 color camera (Zeiss, Switzerland), with Zeiss ZEN 3.8 software, and were processed with the Motic Image Plus 3.0 software. No significant changes in morphology were observed, confirming the safety of all fibrous samples.

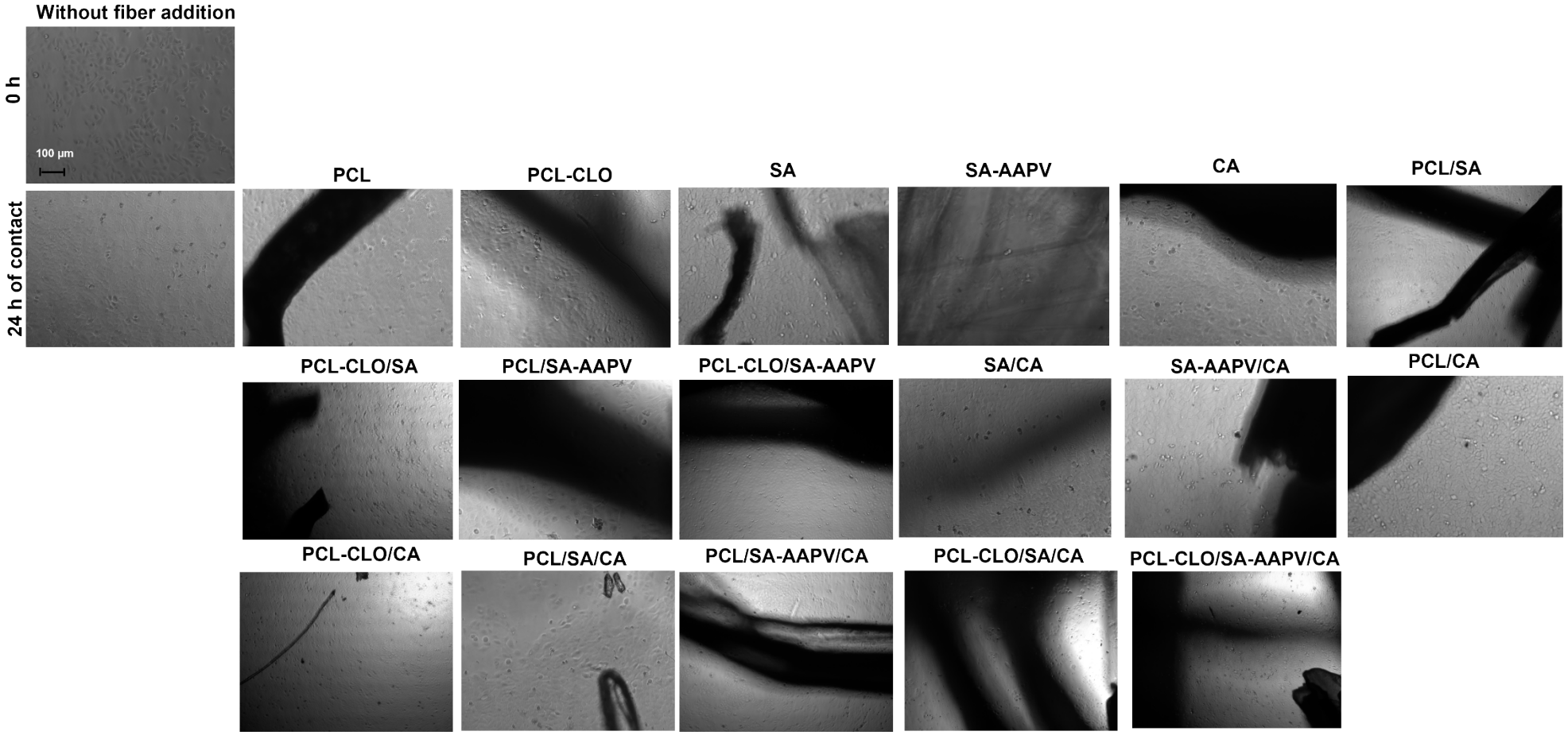

Figure S5. Morphological observations of HaCaT cell lines before and after 24 h of contact with each wet-spun fiber typology, using brightfield microscopy.

The same morphological observations were carried out for NIH 3T3 cell lines (Figure S6).

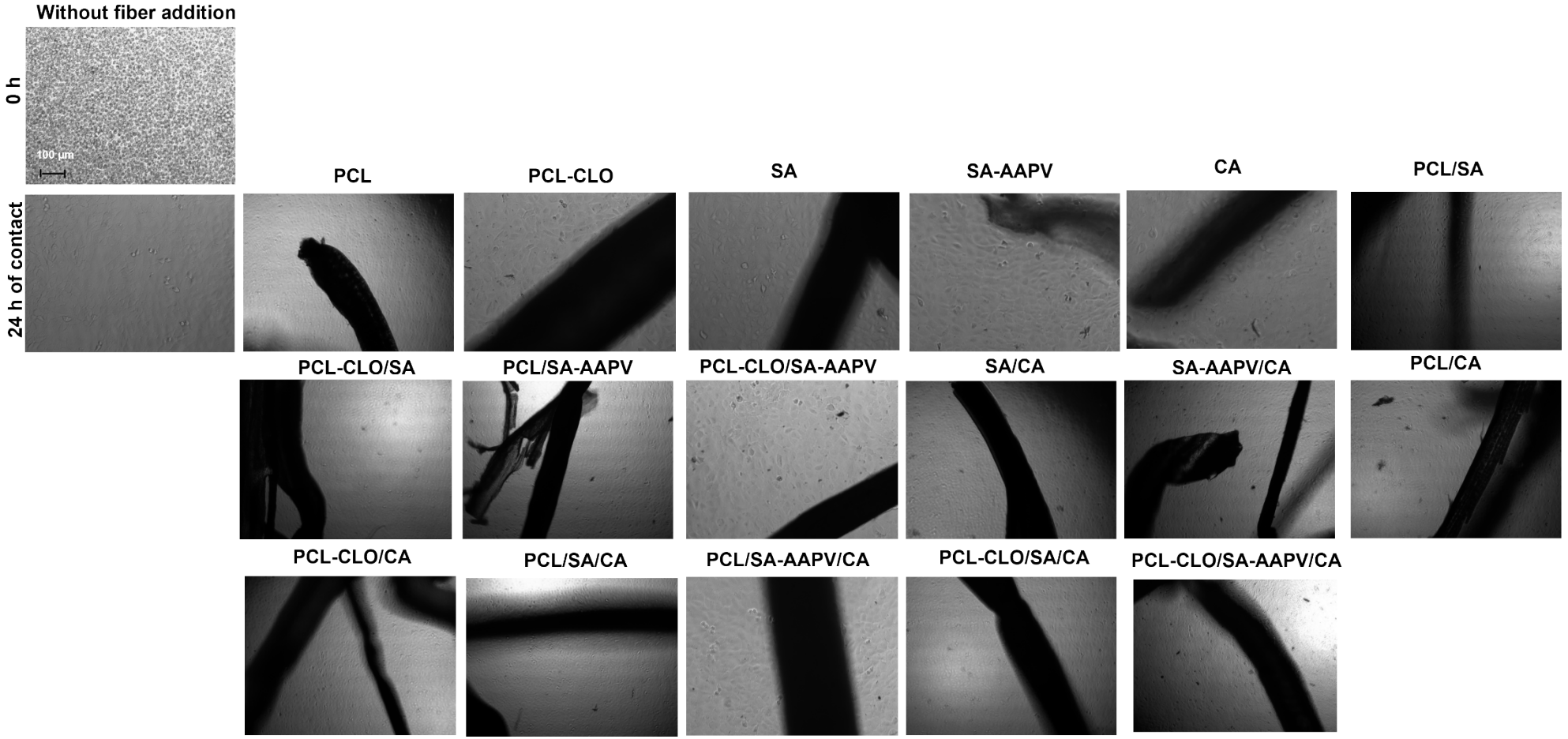

Figure S6. Morphological observations of NIH 3T3 cell lines before and after 24 h of contact with each wet-spun fiber typology, using brightfield microscopy.