

Supplementary Information

Robust and Biocompatible Radiative Cooling Textiles Based on a Synergistic TiO₂ and SiO₂ Nanoparticle Coating

Rayan Ghazi^{†a}, Fatimah Samman^{‡a}, Tadd Truscott^{*b}, Dana AlSulaiman^{*a}

a Materials Science and Engineering Program, Physical Science and Engineering Division, King Abdullah University of Science and Technology (KAUST), Thuwal 23955-6900, Saudi Arabia

b Mechanical Engineering, Physical Science and Engineering Division, King Abdullah University of Science and Technology (KAUST), Thuwal 23955-6900, Saudi Arabia

[†] First author

[‡] Second author

* Emails: tadd.truscott@kaust.edu.sa; dana.alsulaiman@kaust.edu.sa

Content	Pages
Further Experimental Details	2-5
Additional Figures	6-13

1. Further Experimental Details

1.1 Cytotoxicity Assessment

1.1.1 Preparation of Pristine Nanoparticle Dispersions for the CCK-8 Assay

Stock solutions of both TiO₂ and SiO₂ nanoparticle dispersions were prepared at 2 mg·mL⁻¹ in supplemented DMEM. For TiO₂, an additional 1% v/v DMSO was included. The stock dispersions were first diluted in supplemented DMEM to obtain the initial concentrations listed in **Table S 1**. These diluted dispersions were then added to the existing 100 μL of media in the 96-well plate after overnight cell incubation (as described in **Table S 2**), achieving the final concentrations shown in **Table S 1**.

Table S 1: Concentrations of TiO₂ and SiO₂ nanoparticle dispersions and corresponding DMSO content tested in the CCK-8 assay. NP: nanoparticles.

NP Diluted Dispersion	TiO ₂ NP for CCK-8 Assay		SiO ₂ NP for CCK-8 Assay
	Initial Concentration (μg·mL ⁻¹)	Final Concentration in CCK-8 (μg·mL ⁻¹)	DMSO Content in CCK-8 (% v/v)
1000	500	0.25	500
700	350	0.175	350
400	200	0.10	200
200	100	0.05	100
100	50	0.025	50
50	25	0.0125	25
20	10	0.005	10

1.1.2 Preparation of Coated-Cotton Fabrics for Extracts

Table S 2: Preparation steps for the different cotton coatings used in the extract cytotoxicity assay. Green indicates that the step was performed (“yes”), and red indicates it was not performed (“no”). NP: nanoparticles; AA: acrylic acid; PAA: polyacrylic acid.

Preparation Steps	Coating Type					
	Bare Cotton (None)	Non-functionalized TiO ₂ -SiO ₂ NP *	Functionalized PAA	Functionalized TiO ₂ NP	Functionalized SiO ₂ NP	Functionalized TiO ₂ -SiO ₂ NP
Dipping in 70 % ethanol (1 min)	Yes	Yes	Yes	Yes	Yes	Yes
Washing twice in 70 % ethanol	Yes	Yes	Yes	Yes	Yes	Yes
Pretreatment – boiling in deionized water, then soaking (24 h)	Yes	Yes	Yes	Yes	Yes	Yes
Immersion in grafting solution (AA + benzophenone in ethanol, 1 h) with gentle agitation	No	No	Yes	Yes	Yes	Yes
UV irradiation (5 min)	No	No	Yes	Yes	Yes	Yes
Immersion in 0.5 wt % TiO ₂ NP dispersion in ethanol with sonication	No	No	No	Yes	No	No
Immersion in 0.5 wt % SiO ₂ NP dispersion in ethanol with sonication	No	No	No	No	Yes	No
Immersion in TiO ₂ -SiO ₂ NP dispersion (0.5 wt % each) in ethanol with sonication	No	Yes	No	No	No	Yes
Air drying	No	Yes	Yes	Yes	Yes	Yes
Gentle rinsing with 99 % ethanol	No	Yes	Yes	Yes	Yes	Yes
Drying at 70 °C (2h)	No	Yes	Yes	Yes	Yes	Yes
UV disinfection of 3 cm ² cotton pieces ready for extraction (1 h per side)	Yes	Yes	Yes	Yes	Yes	Yes

* The non-functionalized TiO₂-SiO₂ NP coating was washed off after the final ethanol rinse, and the NP were most likely removed from the surface. This was supported by the XPS results (Figure S1 & Table 1), which showed a decrease in the Ti, Si, and O content, and by SEM images (Figure 3), which revealed a non-uniform coating consistent with weak adhesion in the absence of chemical bonding. This was also reflected in the CCK-8 cell viability results (Figure

4), where no significant difference was observed compared to bare cotton. In contrast, the functionalized coatings showed slightly reduced cell viabilities yet remained non-cytotoxic according to ISO 10993-5.

1.1.3 CCK-8 Viability Assay – Detailed Procedure for Nanoparticle Dispersions and Extracts

Table S 3: Detailed steps for performing the CCK-8 assay on nanoparticle dispersions and coated-cotton extracts (in triplicates). Green indicates the step was performed (“yes”), red indicates it was not performed (“no”), gray indicates steps that are not applicable (“NA”), and blue denotes comments. NP: nanoparticles; Double-strength DMEM: DMEM supplemented with 20 % FBS and 2 % penicillin/streptomycin.

Steps	NP Dispersions	Comments	Extracts	Comments
Initial seeding of 8,000 cells/well	Yes	100 µL	Yes	200 µL
Incubation at 37 °C, 5 % CO ₂ (overnight)	Yes	-	Yes	-
Addition of 100 µL treatment/control to existing 100 µL medium	Yes	Treatment: NP concentrations listed in Table S 1 (initial concentration) Control: supplemented DMEM (untreated HDF)	No	-
Removal of existing 100 µL medium	NA	-	Yes	-
Replacement of existing medium with 200 µL treatment/control	NA	-	Yes	Premixed 100 µL of pre-prepared extracts (in non-supplemented DMEM) with 100 µL of <u>double-strength DMEM</u> , yielding extracts at 50 % in normal-strength DMEM to obtain: Treatment: coated-cotton extracts Negative control: supplemented DMEM extract (untreated HDF) Positive control: 20 % v/v DMSO extract
Incubation at 37 °C, 5 % CO ₂ (24 h)	Yes	-	Yes	-
Removal of media and washing with PBS containing Mg ²⁺ & Ca ²⁺	Yes	-	Yes	-
Addition of CCK-8 solution (10 µL CCK-8 reagent in 100 µL supplemented DMEM)	Yes	Include a no cell background for calculation correction	Yes	Include a no cell background for calculation correction

Incubation at 37 °C, 5 % CO₂ (4 h)	Yes	-	Yes	-
Absorbance measurement in a plate reader at 570 nm / 605 nm	Yes	Calculation of cell viability (%)	Yes	Calculation of cell viability (%)

1.1.4 Materials characterization

The crystal structure of the nanoparticles was analyzed using X-ray diffraction (D8 diffractometer, Bruker, USA) with monochromatic Cu K α radiation ($\lambda = 1.5418 \text{ \AA}$), at a scanning step size of 0.02° and a time step of 0.5 s within the 2θ range of 10° – 60° using a $10 \times 10 \text{ mm}^2$ window slit. Structural morphology of all the samples was examined using scanning electron microscopy (SEM, JSM-7600F, Japan). Successful functionalization of the cotton with polyacrylic acid and subsequent nanoparticle coating was evaluated using X-ray photoelectron spectroscopy (XPS).

XPS Survey spectra is given as:

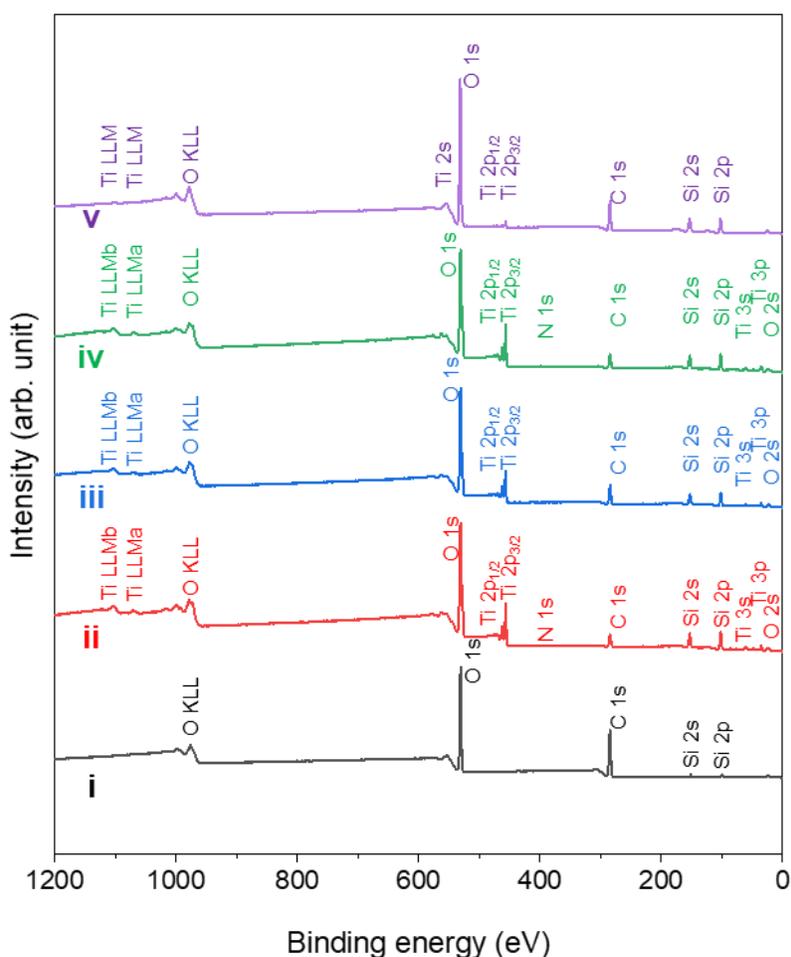


Figure S 1: XPS survey spectrum of (i) bare cotton fabric, (ii) coated cotton fabric before washing, (iii) coating durability of functionalized cotton fabric after washing. (iv) Non-functionalized cotton fabric before washing, and (v) coating durability on non-functionalized cotton fabric after washing, respectively.

2. Additional Figures

2.1 Extract Centrifugation

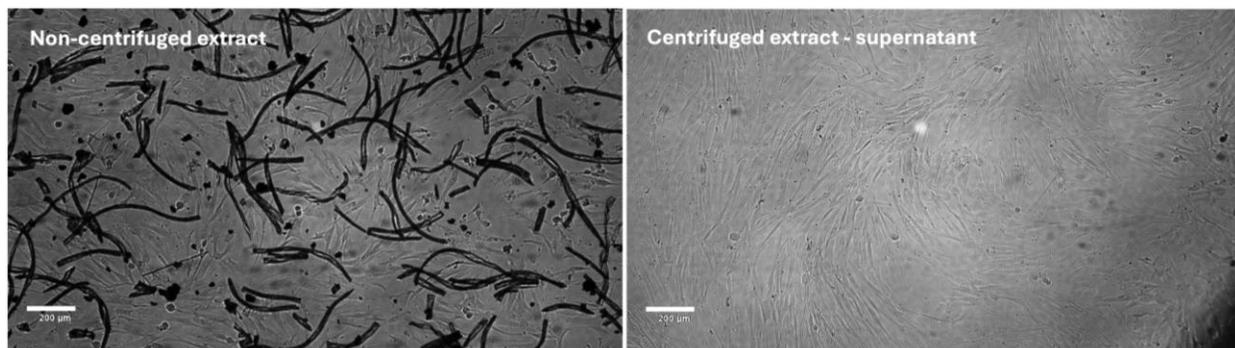


Figure S 2: Camera images showing the effect of centrifugation on loose fabric and large particles in extracts. Extracts that were not centrifuged contained loose fabric and large particles (left), whereas centrifugation at 7300 rpm removed them (right). Centrifugation was performed prior to using the supernatant of extracts to prevent mechanical damage to the cell monolayer that could affect CCK-8 results. Images acquired with Millicell®DCI Digital Cell Imager (Merck) using 10x objective; scale bar: 200 µm.

2.2 *In Vitro* Cytotoxicity of Pristine Nanoparticle Suspensions

2.2.1 Live/Dead Staining of Controls

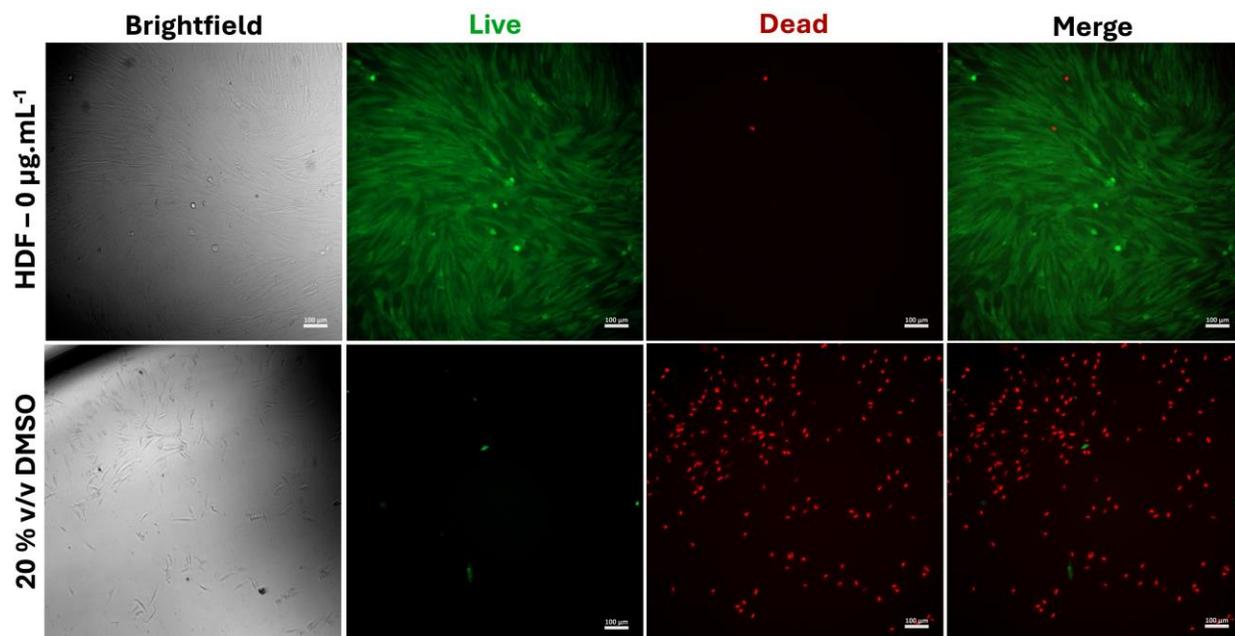


Figure S 3: Live/dead images of HDF cells treated with controls (supplemented DMEM for negative control and DMSO for positive control) for 48 h. Live cells were stained with FDA (cytoplasm) and dead cells with EthD-1 (nuclei). Normal elongated, spindle-shaped morphology is observed in the negative control, whereas few attached cells remain in the DMSO positive control, all of which are dead. Images acquired with an inverted Axio Observer microscope (Zeiss) using 10x objective; scale bar, 100 µm.

2.2.2 Live/Dead Staining of HDF Treated with Pristine Nanoparticle Dispersions at Different Concentrations

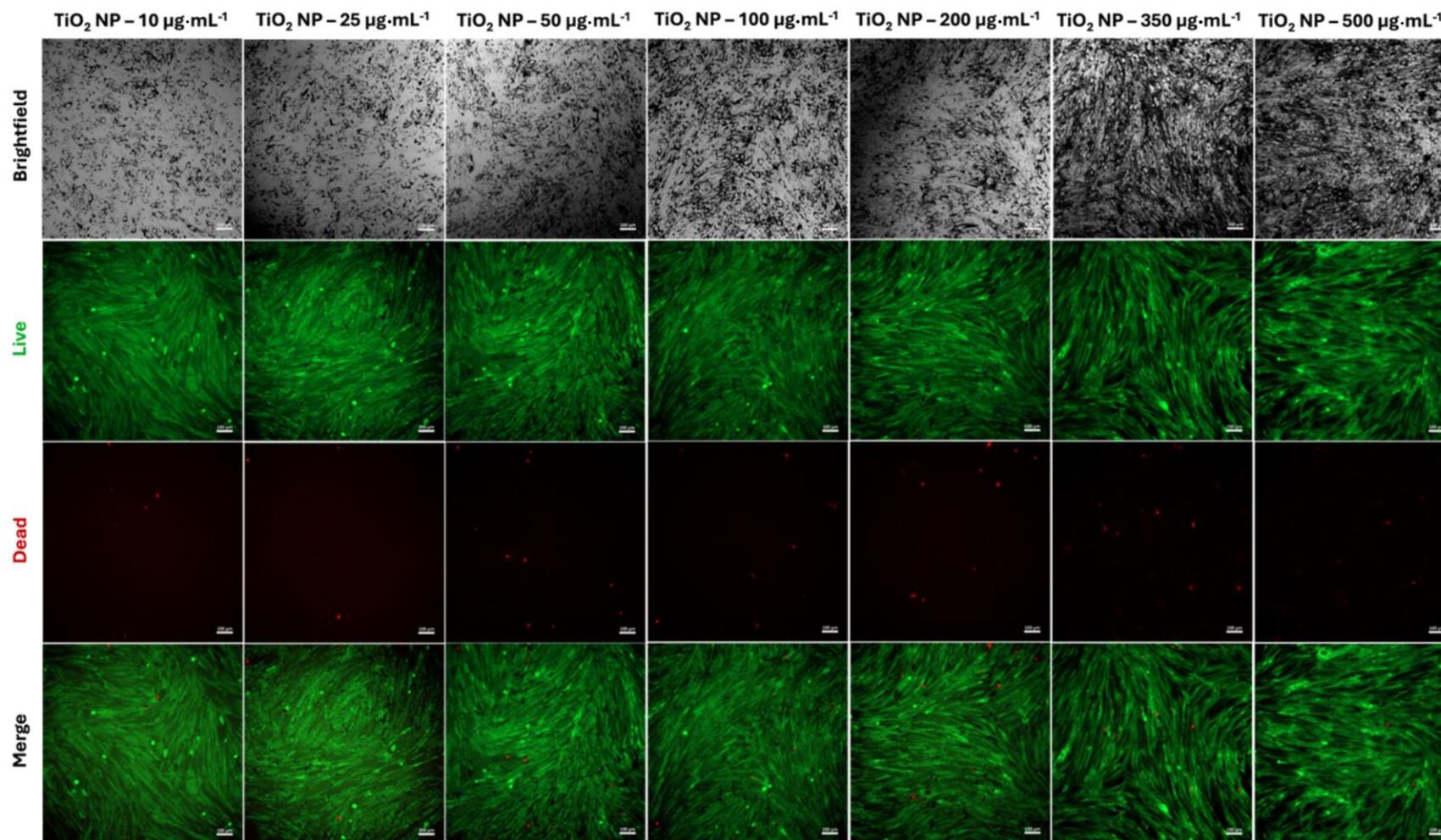


Figure S 4: Live/dead images of HDF cells treated with varying concentrations of TiO_2 nanoparticles for 48 h. Live cells were stained with FDA (cytoplasm) and dead cells with EthD-1 (nuclei). Most cells retained normal elongated, spindle-shaped morphology, with only a few dead cells observed. Nanoparticle clusters appear as black dots within the cytoplasm and around the nucleus in brightfield images. Images acquired with an inverted Axio Observer microscope (Zeiss) using 10x objective; scale bar, 100 μm .

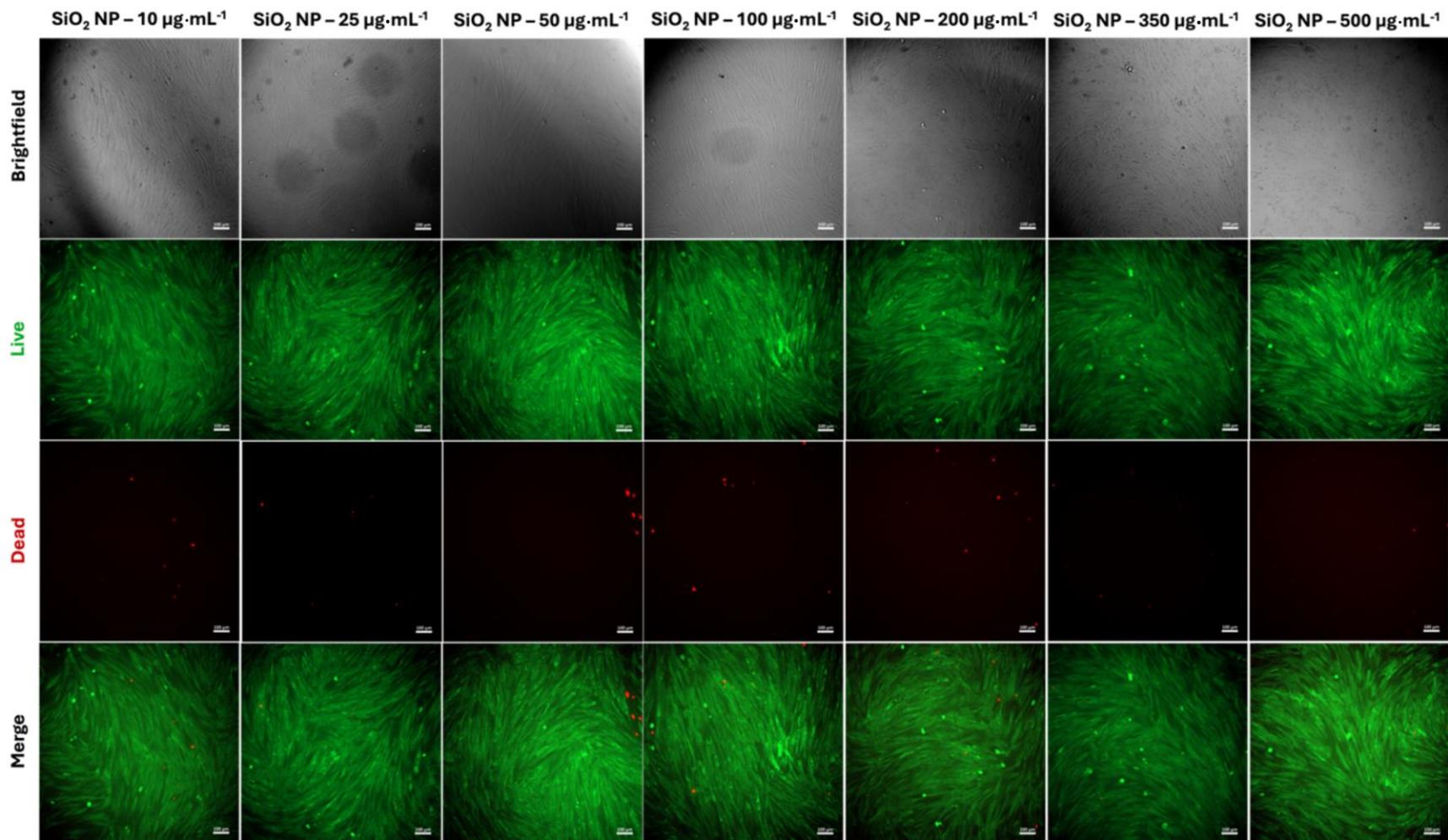


Figure S 5: Live/dead images of HDF cells treated with varying concentrations of SiO₂ nanoparticles for 48 h. Live cells were stained with FDA (cytoplasm) and dead cells with EthD-1 (nuclei). Most cells retained normal elongated, spindle-shaped morphology, with only a few dead cells observed. Nanoparticle clusters appear as black dots within the cytoplasm and around the nucleus in brightfield images at the highest concentrations. Images acquired with an inverted Axio Observer microscope (Zeiss) using 10x objective; scale bar, 100 µm.

2.2.3 Morphological Studies of HDF Treated with Pristine Nanoparticle Dispersions at Different Concentrations

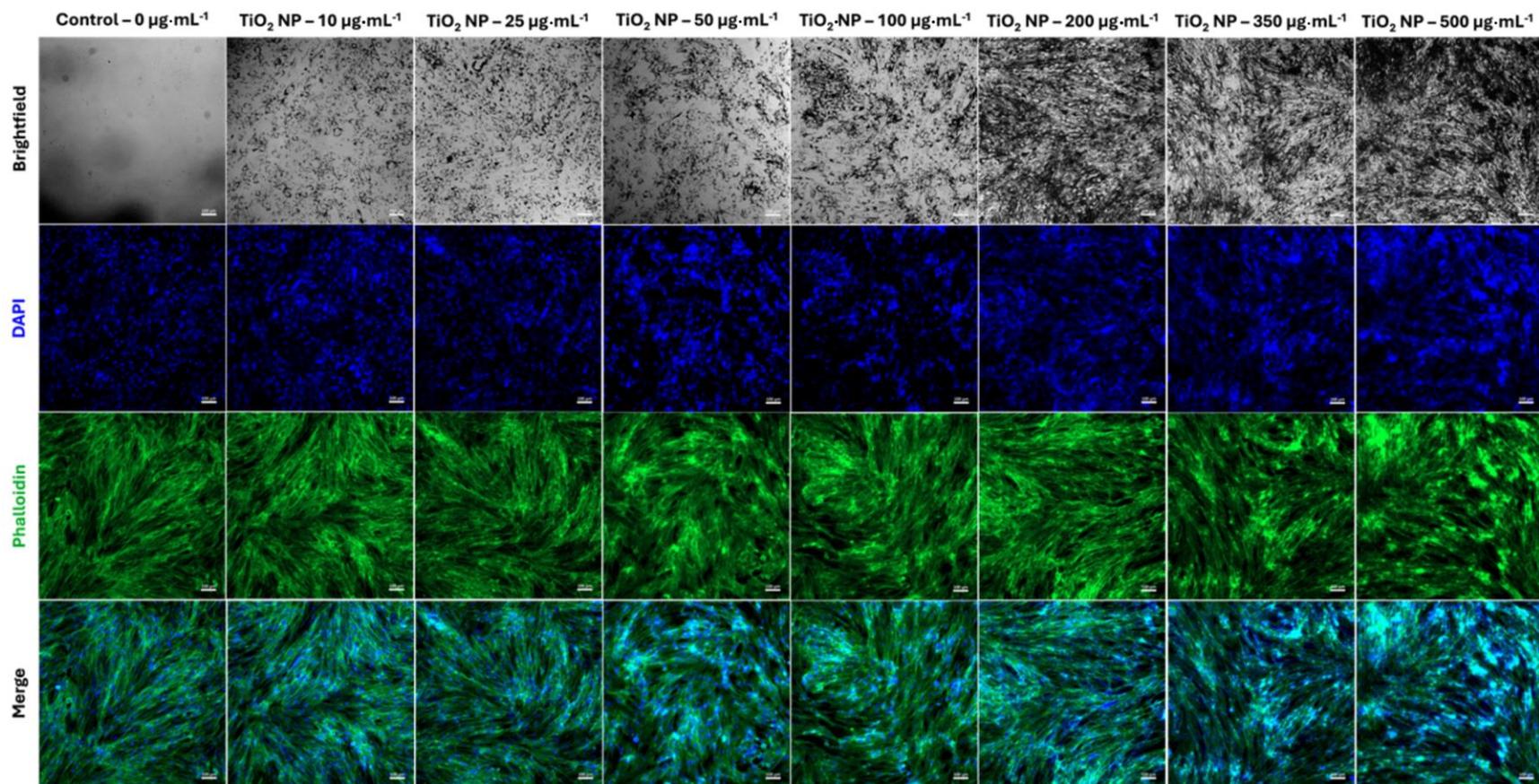


Figure S 6: HDF cells treated with varying concentrations of TiO₂ nanoparticle dispersions for 48 h. Cells retained normal elongated morphology at all concentrations, comparable to untreated controls. Nanoparticle clusters appear as black dots within the cytoplasm and around the nucleus in brightfield images. DAPI and phalloidin were used for the nuclei and cytoskeleton F-actin filaments, respectively. Images acquired with an inverted Axio Observer microscope (Zeiss) using 10x objective; scale bar, 100 µm.

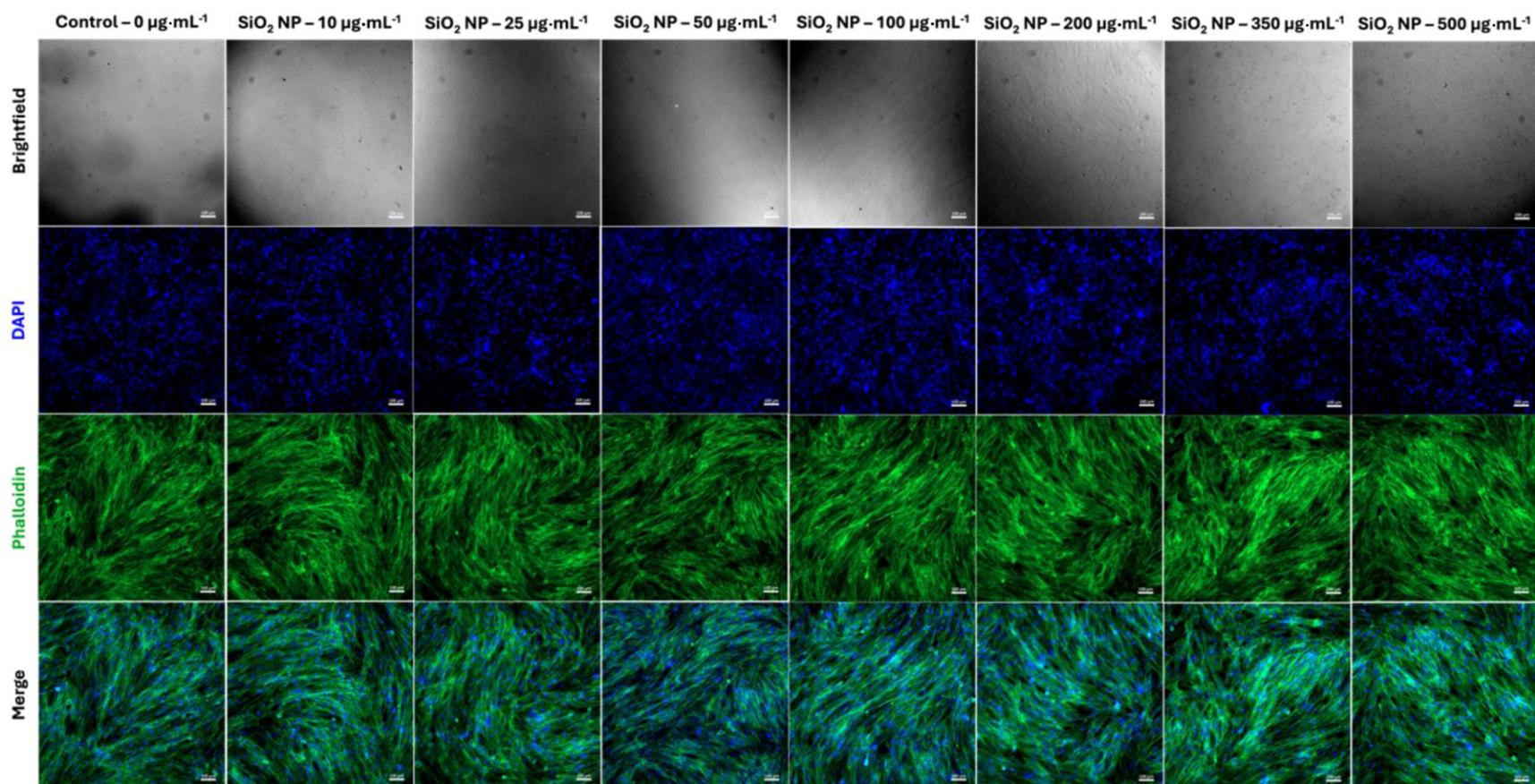


Figure S 7: HDF cells treated with varying concentrations of SiO₂ nanoparticle dispersions for 48 h. Cells retained normal elongated morphology at all concentrations, comparable to untreated controls. Nanoparticle clusters appear as black dots within the cytoplasm and around the nucleus in brightfield images. DAPI and phalloidin were used for the nuclei and cytoskeleton F-actin filaments, respectively. Images acquired with an inverted Axio Observer microscope (Zeiss) using 10x objective; scale bar, 100 μm .

2.3 In Vitro Cytotoxicity of Extracts

2.3.1 Extracts Sterility on LB Agar Plate

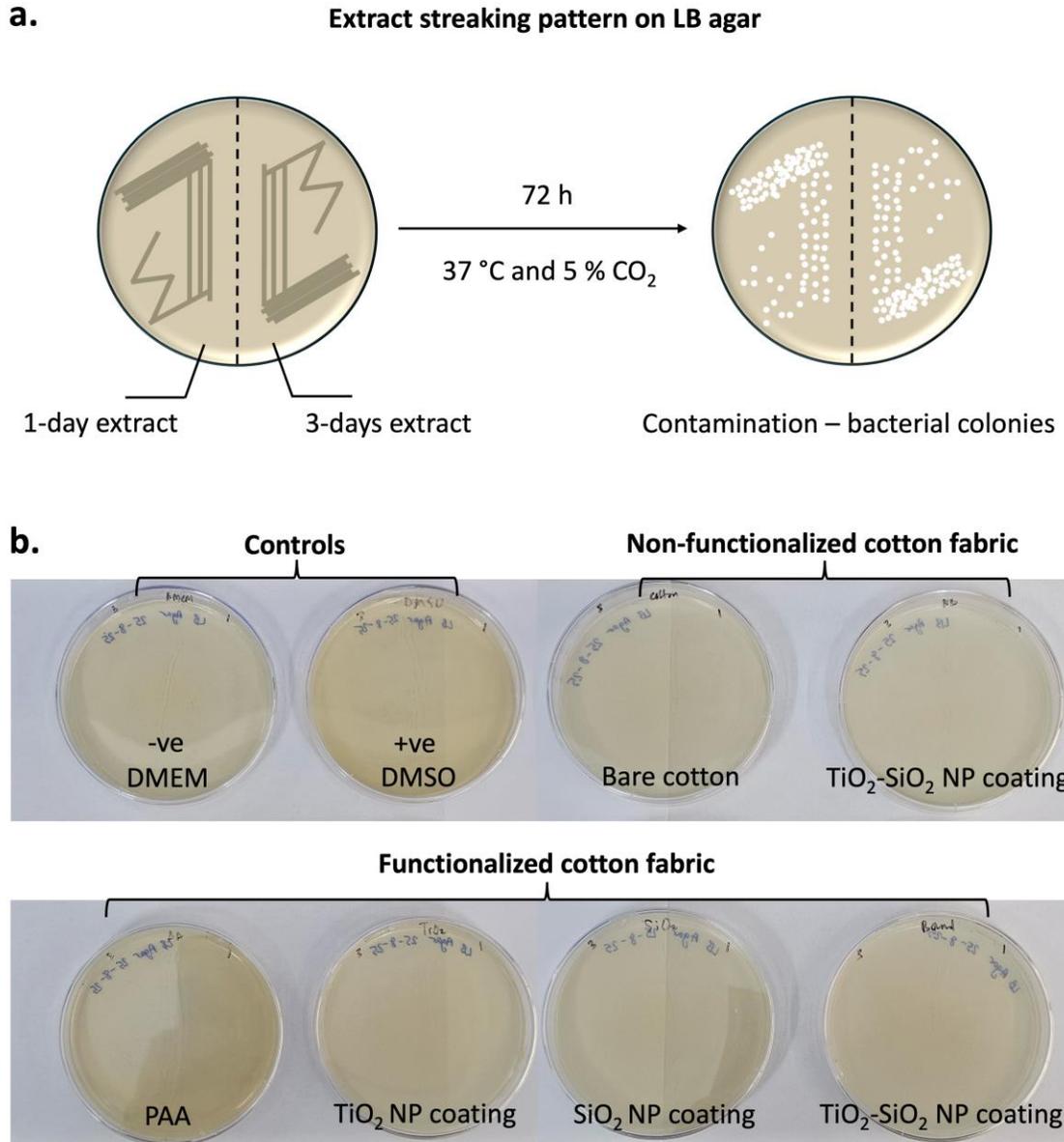


Figure S 8: (a) Schematic illustrating the streaking of extracts onto an LB agar plate to assess sterility. A 20 μ L aliquot of each extract was streaked along the indicated lines, from the dense to less dense areas (left). Contaminated extracts would show bacterial growth along the streaks (right), whereas sterile extracts remain free of growth and are ready for use. (b) All extracts were confirmed sterile after incubation on LB agar for 72 h at 37 °C and 5% CO₂, as no bacterial growth was observed.

2.3.2 Cellular Morphology of HDF Treated with Extracts – Digital Cell Imager

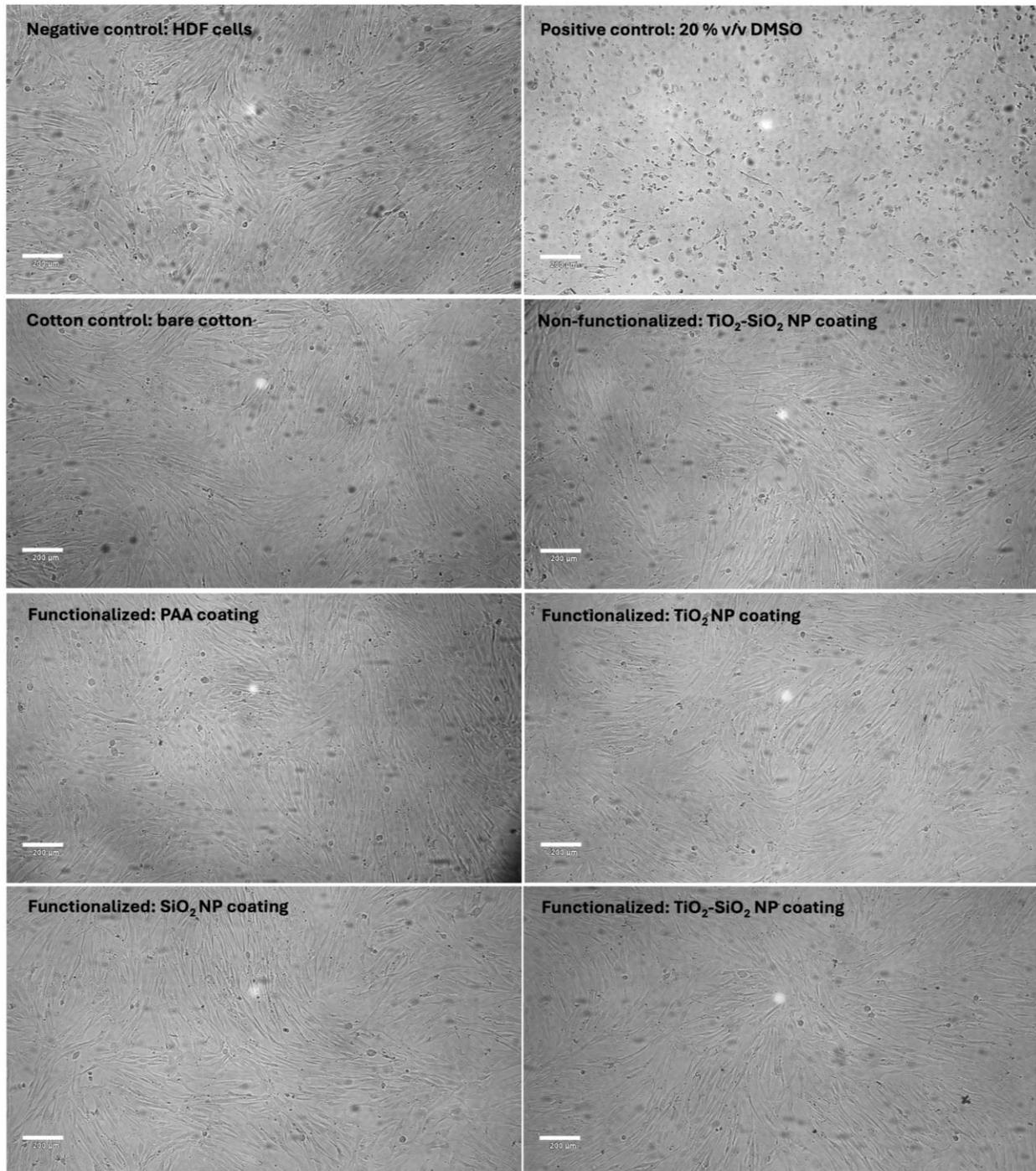


Figure S9: HDF cells treated for 24 h with 1-day extracts at 37 °C. Cells exposed to bare cotton or coated-cotton extracts retained normal spindle-shaped morphology, forming elongated, well-spread monolayers with clear boundaries, comparable to non-treated control. In contrast, 20 % v/v DMSO-extract-treated cells were mostly rounded, detached, and showed extensive debris. Images acquired with Millicell®DCI Digital Cell Imager (Merck) using 10x objective; scale bar: 200 µm.

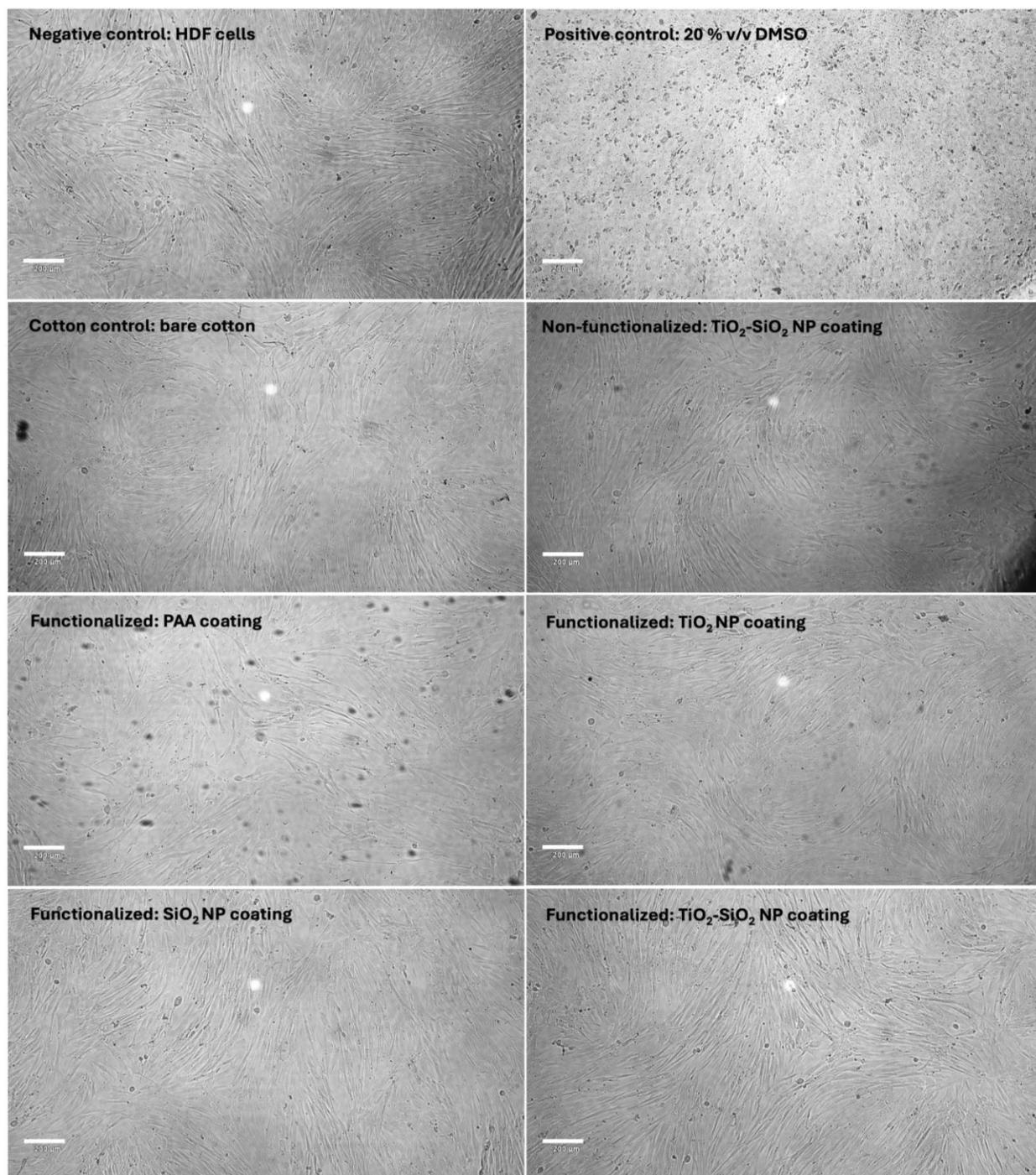


Figure S 10: HDF cells treated for 24 h with 3-day extracts at 37 °C. Cells exposed to bare cotton or coated-cotton extracts retained normal spindle-shaped morphology, forming elongated, well-spread monolayers with clear boundaries, comparable to non-treated control. In contrast, 20 % v/v DMSO-extract-treated cells were mostly rounded, detached, and showed extensive debris. Images acquired with Millicell®DCI Digital Cell Imager (Merck) using 10x objective; scale bar: 200 µm.