Supplementary Information for:

Nanostructured fibrous membranes with rose spike-like architecture

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Materials

Poly ε-caprolactone (PCL) with an average molecular weight of 80,000, and 1,1,1,3,3,3-hexafluoroisopropanol (HFIP) was purchased from Sigma-Aldrich (St. Louis, MO, USA). Cell culture supplies including Dulbecco's modified Eagle medium (DMEM), 0.05% trypsin-EDTA (10X), fetal bovine serum (FBS), antibiotics, fibronectin, and PrestoBlue® Assay were purchased from Invitrogen (Carlsbad, CA, USA).

Branched ZnO Nanoparticle Synthesis

To produce branched ZnO nanoparticles, a flame transport synthesis technique was used. This method offered direct conversion from metallic Zn microparticles into complex shaped ZnO nano- and microstructures in a single step conversion within the flame in the presence of normal air environment. The mixture (2:1 weight ratio)of sacrificial polyvinylbutyral polymer and / or ethanol and Zn microparticles from Goodfellow, UKwas burned in a simple muffle type oven where Zn particles were directly converted into branched ZnO nanostructures via solid-vapor-solid growth in a further modified flame transport synthesis process, as described in a previous work[1].

Electrospinning Zinc Oxide Composites

400 mg of PCL (Mn 80,000) was added to a scintillation and 4 mL of HFIP solvent to create a 10 % (w/v) solution, which was stirred overnight. To disperse the particles, ZnO was added to the polymer solution and bath sonicated for 30-50 min prior to electrospinning. The composite was then added to a 3 mL syringe, attached to a 23-gauge blunt-tip needle. The polymer was extruded at a flow rate of 2 mL/h, and the grounded electrode was placed 25 mm below the needle. A voltage in the range of 17.0 to 18.0 kV was applied to generate the fibrous substrates. During the

electrospinning process, the relative humidity and temperature were set at 15% and 26° C respectively.

Optical and Elemental Analysis of Zinc Oxide Composites

The nanocomposite fiber morphology was investigated using a scanning electron microscope (SEM; Zeiss Ultra Plus, 7 kV) with an energy–dispersive X-ray (EDX) spectrometer. The nanocomposite fibers were sputtered with a thin gold layer to reduce the surface charge; subsequently compositional analysis was performed merging the SEM data.

Fourier Transformer Infrared Spectroscopy (FTIR)

Nanocomposite structure absorbance spectra were obtained by a Bruker Alpha FTIR (Bruker Optic GmbH, Ettlingen, Germany) using attenuated total reflection (ATR) configuration. The machine was tarred by running a blank sample to subtract the background absorbance. Electrospun samples where then loaded into the instrument, and absorbance spectra were detected.

X-ray Fiber Diffraction

The electrospun samples were sliced to obtain 2 mm x 10 mm ribbons, and then were carefully mounted and aligned, with their long ribbon axis vertical, onto the Cu monochromatic X-ray beam using an Oxford XCalibur PX Ultra diffractometer equipped with a low noise CCD Onyx area detector. All experimental conditions such as time of scanning θ, χ, theta, chi, phi Kappa diffractometer were keep constant for all the tested conditions. The detector distance was calibrated using the sharp 104 reflections at 3.035 Å of $CaCO_3$

Tensile Experiment

The tensile properties were calculated using a uniaxial tensile machine (Instron, Norwood, MA) with cell load capacity of 10 N setting the extension rate at 15 mm/min. The specimens were cut from the mat sheets in order to obtain standard dimension: 3.5 mm width, 9.5 mm length, 400 μm thickness. The stress-strain curves were plotted (n=4) and the elastic modulus was derived from the initial 0-10% linear region of the stress-strain curves.

Lap Shear Adhesion Test

The adhesive properties of electrospun were analyzed using an ASTM standard lap shear test (F2255-05). Briefly, the fiber was sandwiched in between a piece of porcine skin (24.0 mm width, 15.0 mm length, and 3.0 mm height). A small 500-gram weight was placed on top of the sandwiched fibers for 1 min then immediately tested until failure in a lap shear setup using an Instron 5542 mechanical tester equipped with a 10 N load cell at a cross-head speed of 5 mm/min.

Contact Angle Measurements of Fiber Composites

Samples containing 1, 3, and 5% (w/v) ZnO were electrospun onto a glass slide to retain flat surfaces. The slide was deposited with a homogenize layer of fibers. The contact angle measurement (n=6) was performed by a contact measurement system (KSV CAM 101) at room temperature, and 20% relative humidity. The machine was calibrated before measurements to insure quality of measurements. A water droplet volume of 29 μL was placed on the fibrous surface and the droplet profile was captured with the camera, finally contact angles were measured.

Bacteria Culture and Antimicrobial Studies

Control (pristine PCL), spherical, and branched samples containing 1% (w/v) particles were placed in separate wells of a 24 well plate, and sterilized under UV light. *Pseudomonas aeruginosa* and *Escherichia coli* were used to evaluate the antimicrobial properties of the electrospun sheets. A single colony of each strain of bacteria was mixed in 5 mL of tryptic soy broth (TSB, Sigma-Aldrich), and incubated overnight in a shaker incubator (200 rpm at 37 °C). The optical density of the resulting bacterial suspension was adjusted to 562 nm, which corresponds to a density of 10^9 CFU/ml. This suspension was then serially diluted to a density of 10^6 CFU/mL. Subsequently, 1 ml of the bacterial suspension was added directly on top of each sample and incubated at 37 °C and 5% CO_2 for 24 h. After incubation, the scaffolds were carefully washed 3 times with PBS to remove excess bacteria. For CFU assays, the scaffolds were placed in 1 mL DPBS in 1.5 mL microcentrifuge tubes. The tubes were vigorously vortexed (3000 rpm) for 15 min to release all bacteria from the scaffold into the solution. Each bacterial suspension was serially diluted in DPBS over 4 logarithmic dilutions. Then, three 10 μL drops of each dilution were seeded on tryptic soy agar plates, which were then incubated for 24 hr. at 37 °C and 5% CO_2. Lastly, the number of bacterial colonies formed on each agar plate was counted, and the dilution factor was used to calculate CFU values.

Cell Culture and Cell Viability Methods

Human keratinocytes were grown to confluence in Dulbecco's modified eagle medium, supplemented with 10% (v/v) FBS and 1% (v/v) streptomycin-penicillin maintained at 37 °C in a 5% CO_2 environment. Cells with a passage number between 6-8 were used for the experiments. Fibrous structures with a diameter of 5 mm were sterilized with 70% ethanol and washed with

antibiotic-antimycotic solution and DPBS. Prior to cell seeding, the fibers were coated with 10 ug/mL of fibronectin for 2 h. Cells were seeded at a concentration of 1.875 x 10^6 cells/mL. In vitro metabolic activity of the cells was analyzed after 1 and 3 days of culture with PrestoBlue® assay as per manufacturer's instructions. Samples fluorescence was recorded at 560 nm (excitation) and 590 nm (emission) using a microplate reader (BioTek synergy™ 2, USA).

Statistical Analysis

The results were reported as the mean ±standard deviation. Graphpad Prism version 7 was used to perform one-way ANOVA with Tukey post hoc tests to determine the significance of the differences between groups (*: $P<0.05$, **: $P<0.002$, ***: $P<0.001$).

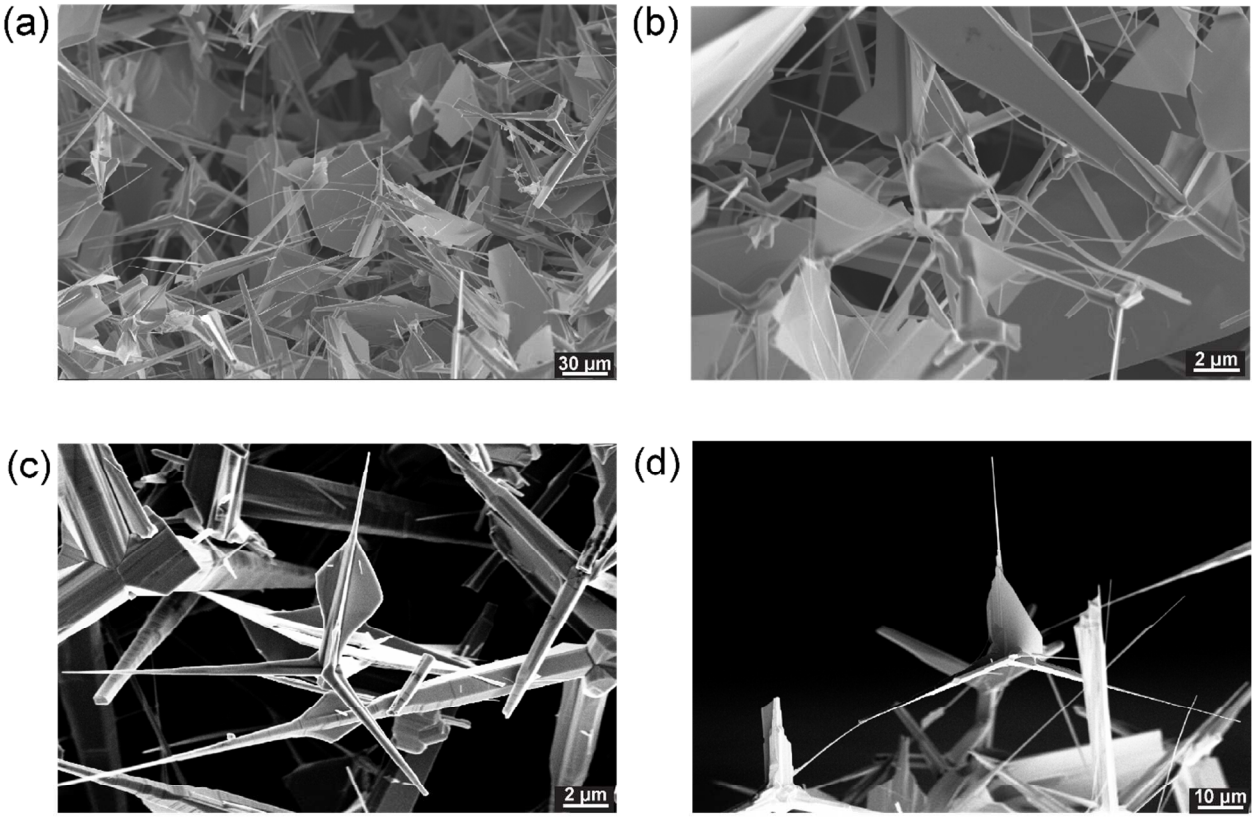

Figure S1. SEM imaging of pristine branched ZnO. (a-d) SEM images of branched ZnO nanoparticles produced *via* modified flame transport synthesis

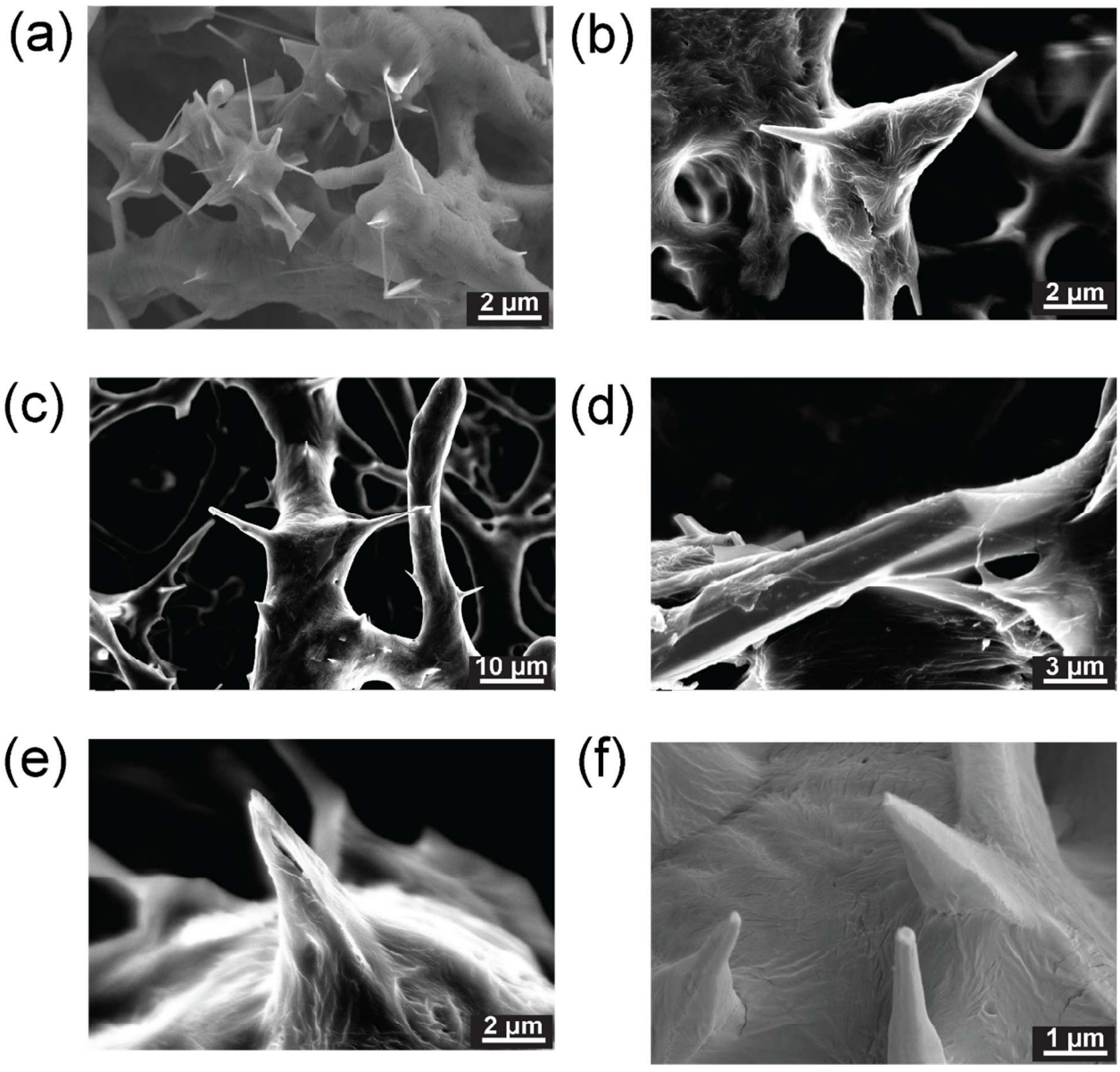

Figure S2. SEM imaging of composite branched ZnO fibers. (a-f) SEM analysis of branched ZnO nanoparticles composite fibers with varying magnification.

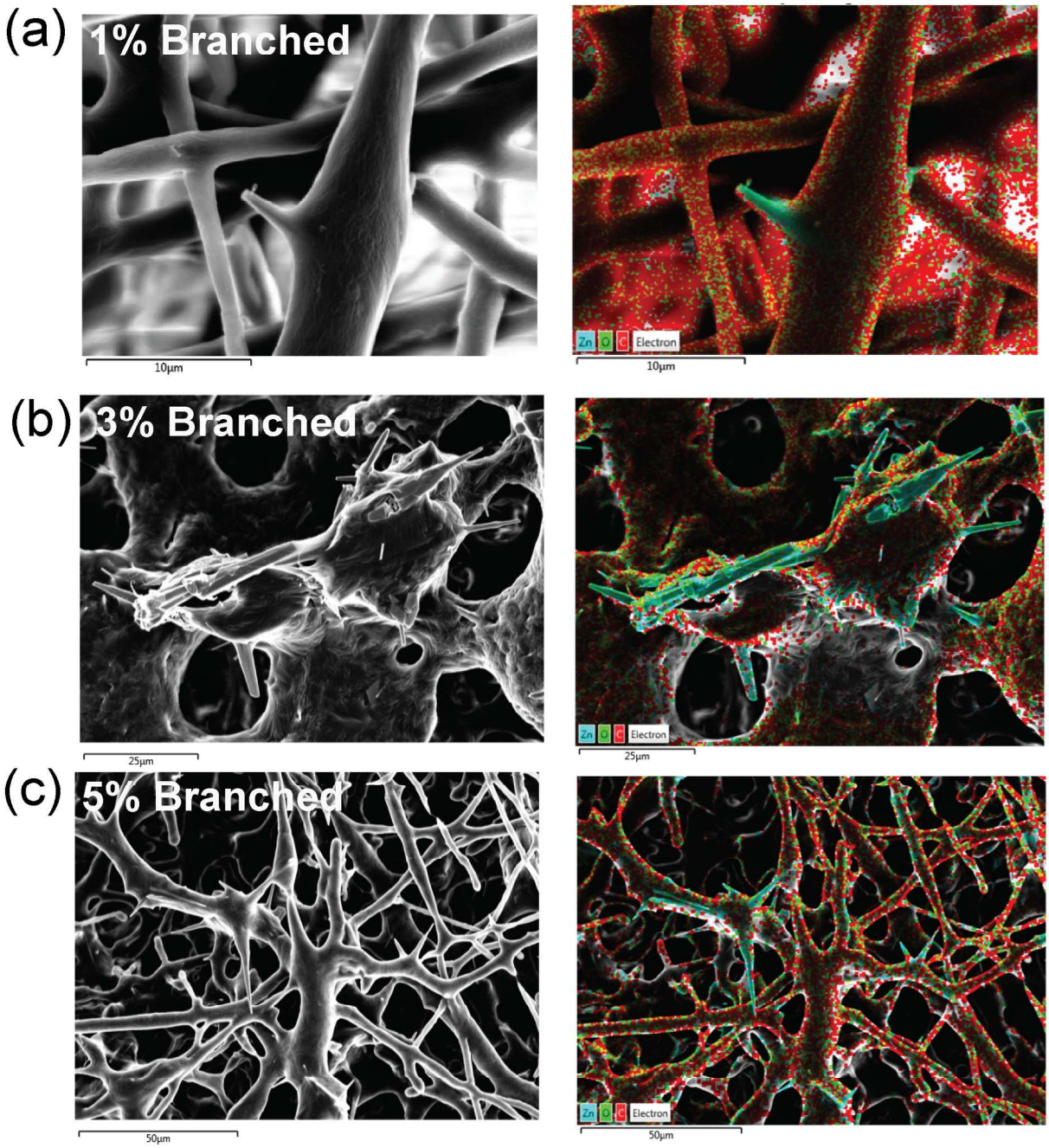

Figure S3. EDAX imaging of composite branched ZnO fibers. (a) 1% w/v Branched Composite (b) 3% w/v Branched Composite (c) 5% w/v Branched Composite

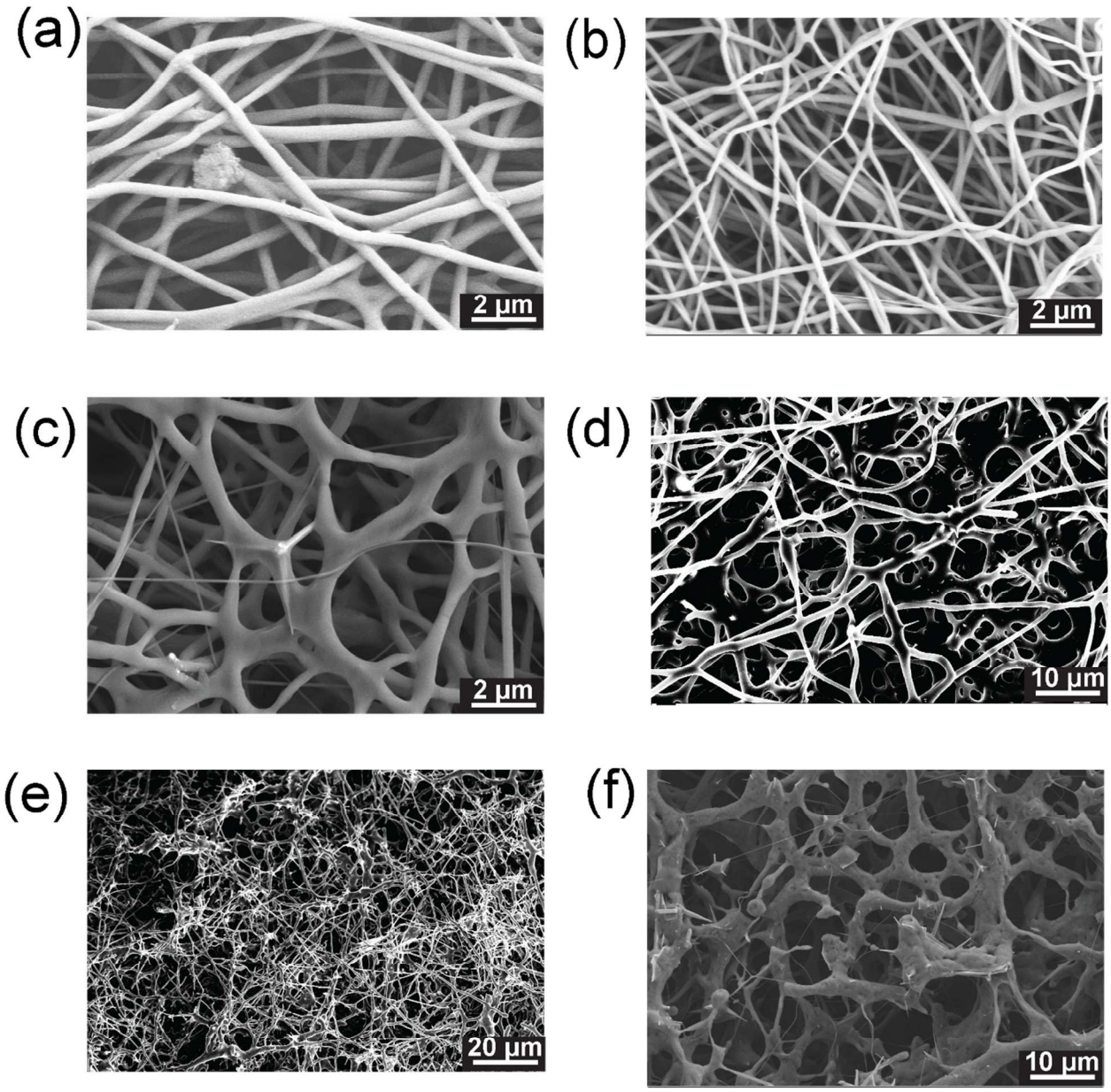

Figure S4: Low Magnification SEM image of engineered membranes. (a, b) PCL fiber morphology (c,d) 3% (w/v) branched particles (e,f) 5% (w/v) branched particles

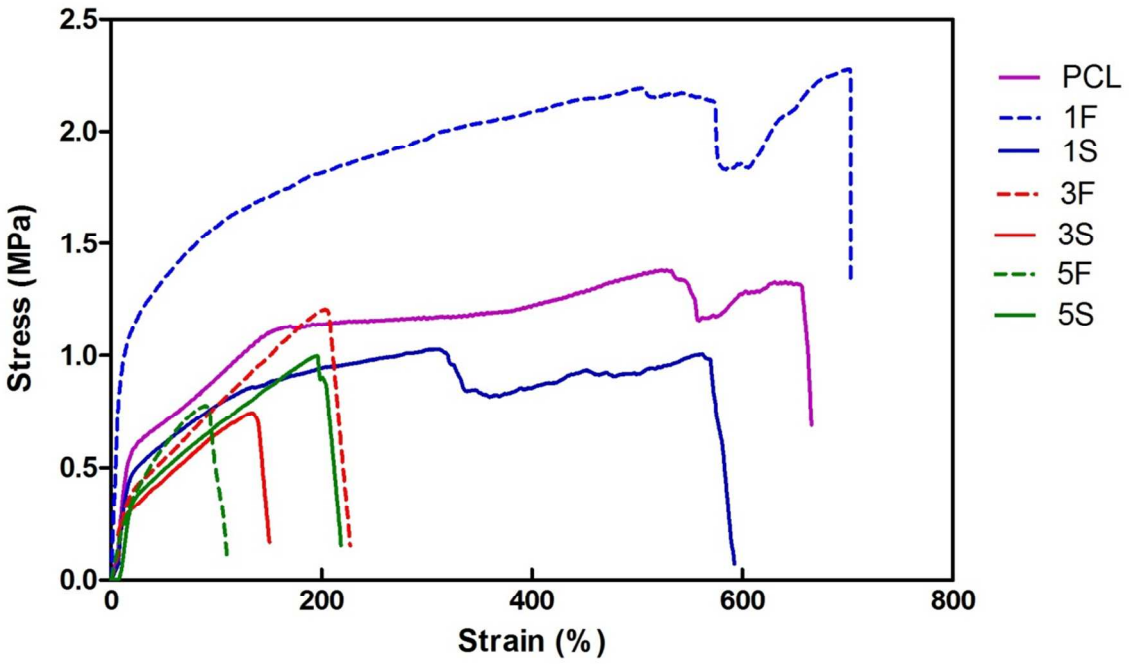

Figure S5: Stress-Strain curve. Tested scaffolds with varying particle concentrations and shape tested (branched versus spherical ZnO particles).

Supplementary References:

1. Mishra, Y. K.; Kaps, S.; Schuchardt, A.; Paulowicz, I.; Jin, X.; Gedamu, D.; Freitag, S.; Claus, M.; Wille, S.; Kovalev, A.; Gorb, S. N.; Adelung, R. *Part. Part. Syst. Charact.* **2013,** 30, (9), 775-783.