Antibacterial Porous Electrospun Fibers as Skin Scaffolds for Wound Healing Applications

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DMSO stocks preparation

Overnight culture was diluted 100 times in fresh LB medium (100 µL on bacteria in 10 mL medium) and grown at 37°C aerobically to an exponential phase. At optical density of 0.8 (OD600), dimethyl sulfoxide (DMSO) was added to a final concentration of 8%. 120 µL of suspension was added to PCR tubes and frozen in liquid nitrogen. The tubes were stored in - 80°C and used within 6 months.

Bacterial attachment, growth and bacterial biofilm formation

Overnight liquid culture of bacteria in LB was grown from DMSO stocks. The culture was diluted to about $5x10^7$ colony-forming units (CFU)/mL with DMEM supplemented with 10% heat-inactivated FBS. 1 mL of the bacterial dispersion was added to 1 cm^2 square-shaped samples in 24 well-plates. The well-plates were incubated at 37°C for 24, 48 or 72 h. After that the samples were rinsed twice with 1 x PBS and put into 1 mL of fresh 1 x PBS in 1.5 ml Eppendorf tube. For disrupting the biofilm alternating vortexing (Vortex-Genie 2, Scientific Industries) and sonication (Bandelin Sonorex digital 10 P, operating at 20% of maximum power) was performed in 30 s cycles. Each cycle was repeated 6 times, as this was seen to provide the best compromise between biofilm disruption and bacterial viability. The CFUs were determined by making 10-times dilutions of the dispersion, plating these as 5 µL drops on LB agar plates and counting the CFUs at optimal dilutions after 18 h of incubation. Planktonic bacteria in each time point were always plated as controls in order to verify the growth of bacteria in each condition. A minimum of three technical replicates were performed.

Characterization of surface pores on electrospun fibers

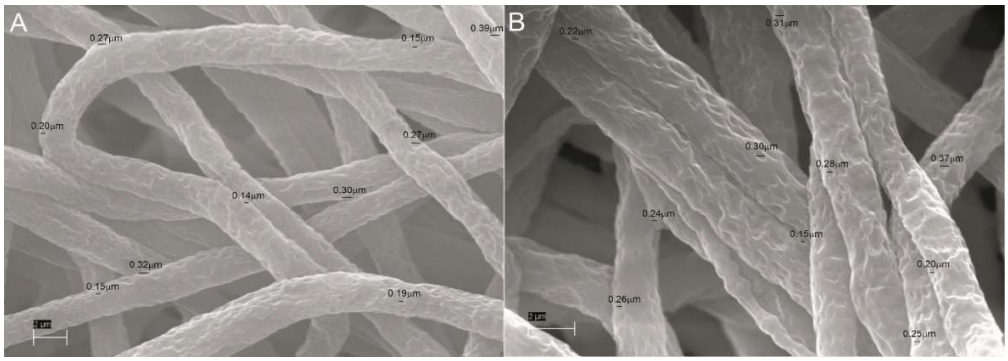

Figure S1. SEM micrographs of surface pore analysis for A. Porous microfibers and B. Porous microfibers with CAM. (Magnification 10000x).

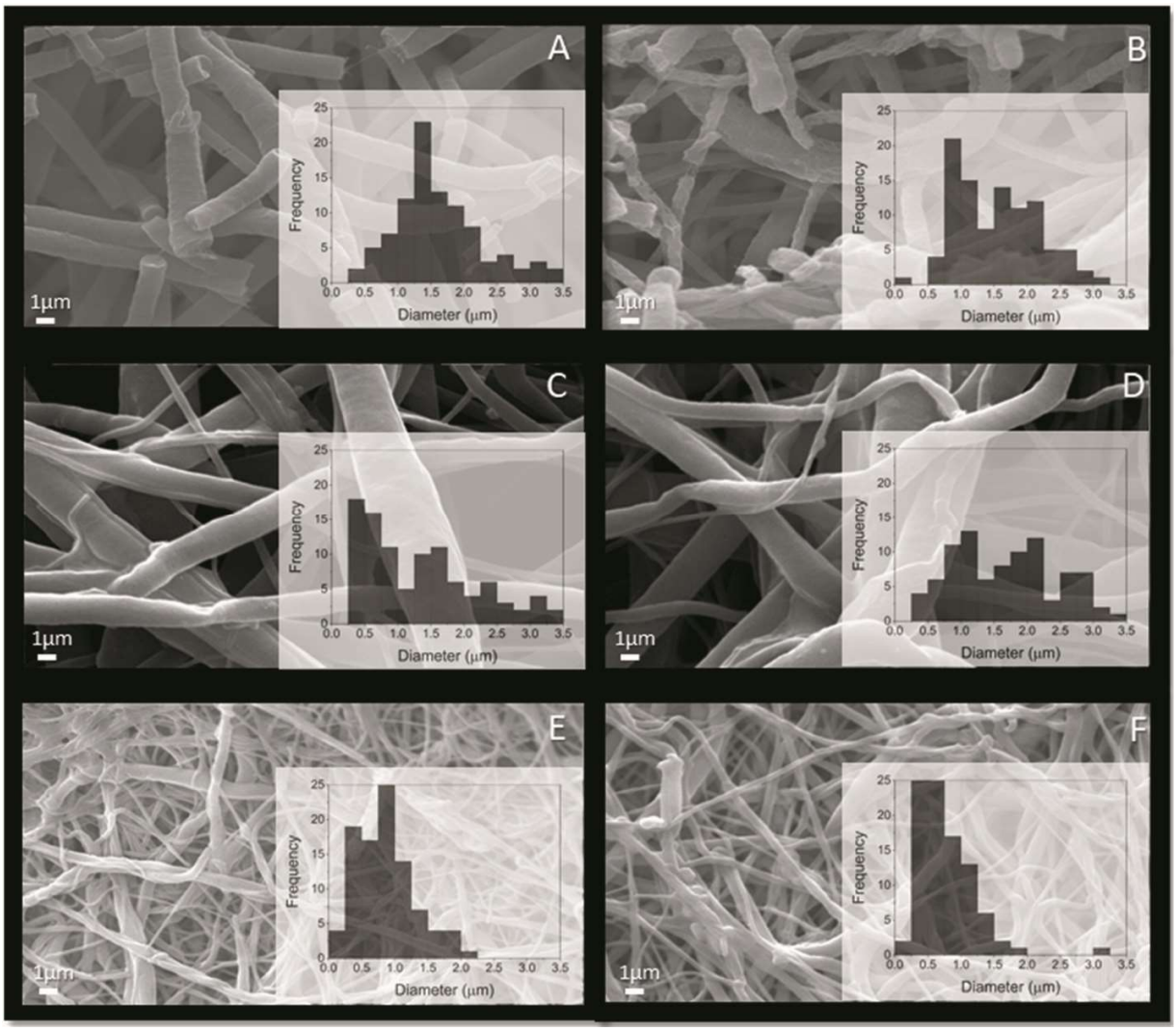

Figure S2. SEM micrographs after swelling experiment (24 h at 37°C in 1 x PBS). A – porous microfibers; B – porous microfibers with CAM; C – non-porous microfibers; D- non-porous microfibers with CAM; E – non-porous nanofibers; F- non-porous nanofibers with CAM.

Cell adhesion and migration into the scaffold structure

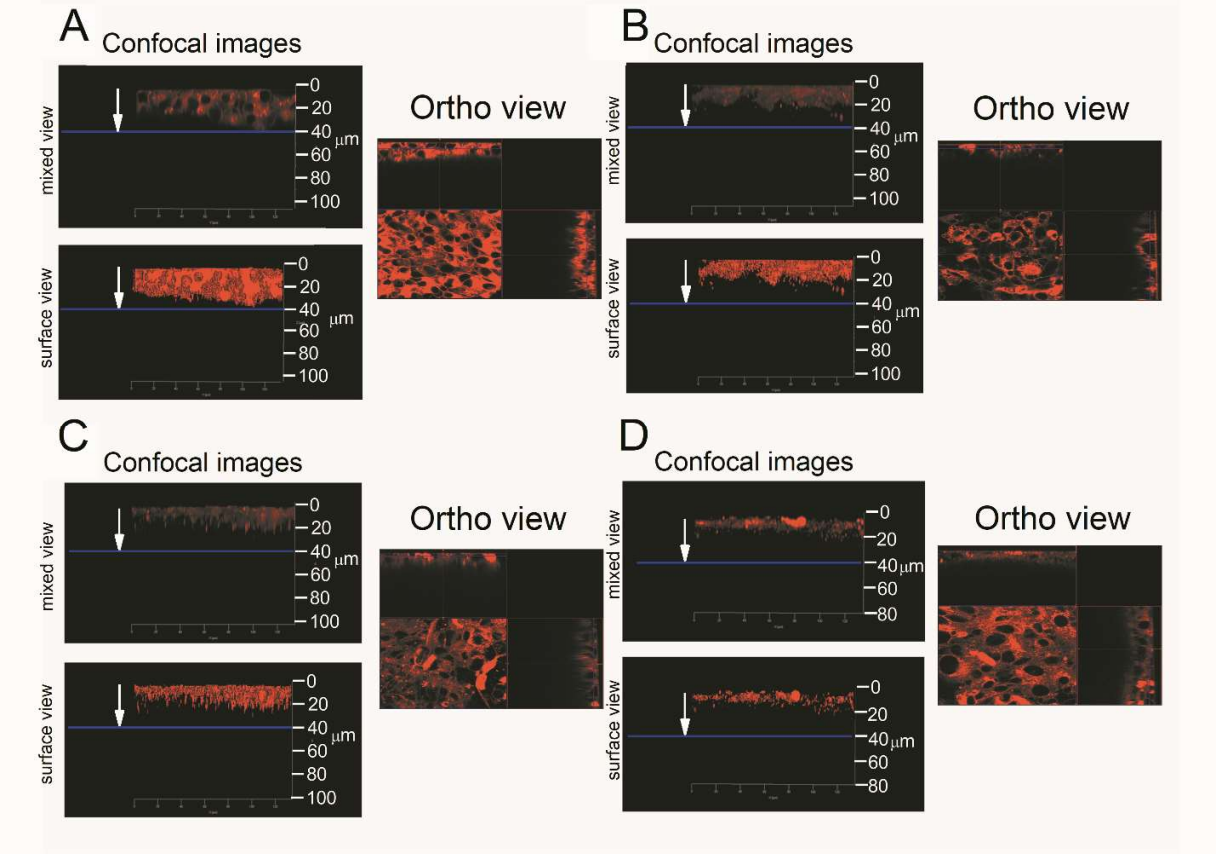

Figure S3. Confocal fluorescent microscopy (CFM) images fibroblast cells (BHK-21) attached onto electrospun **A.** porous PCL microfiber scaffold consisting CAM; **B**. non-porous PCL microfiber scaffold; **C.** non-porous PCL nanofiber scaffold; **D.** non-porous PCL nanofiber scaffolds consisting CAM. Cross-section micrographs (mixed and surface view) and orthographic projection (ortho view) of 3D images are shown. The white arrows on Figures **C,D,E** indicate the fibroblast infiltration from 0 – 40 µm (blue line) in depth. The pictures were constructed from 101 slices of z-stack images with the depth of 134.95 µm.

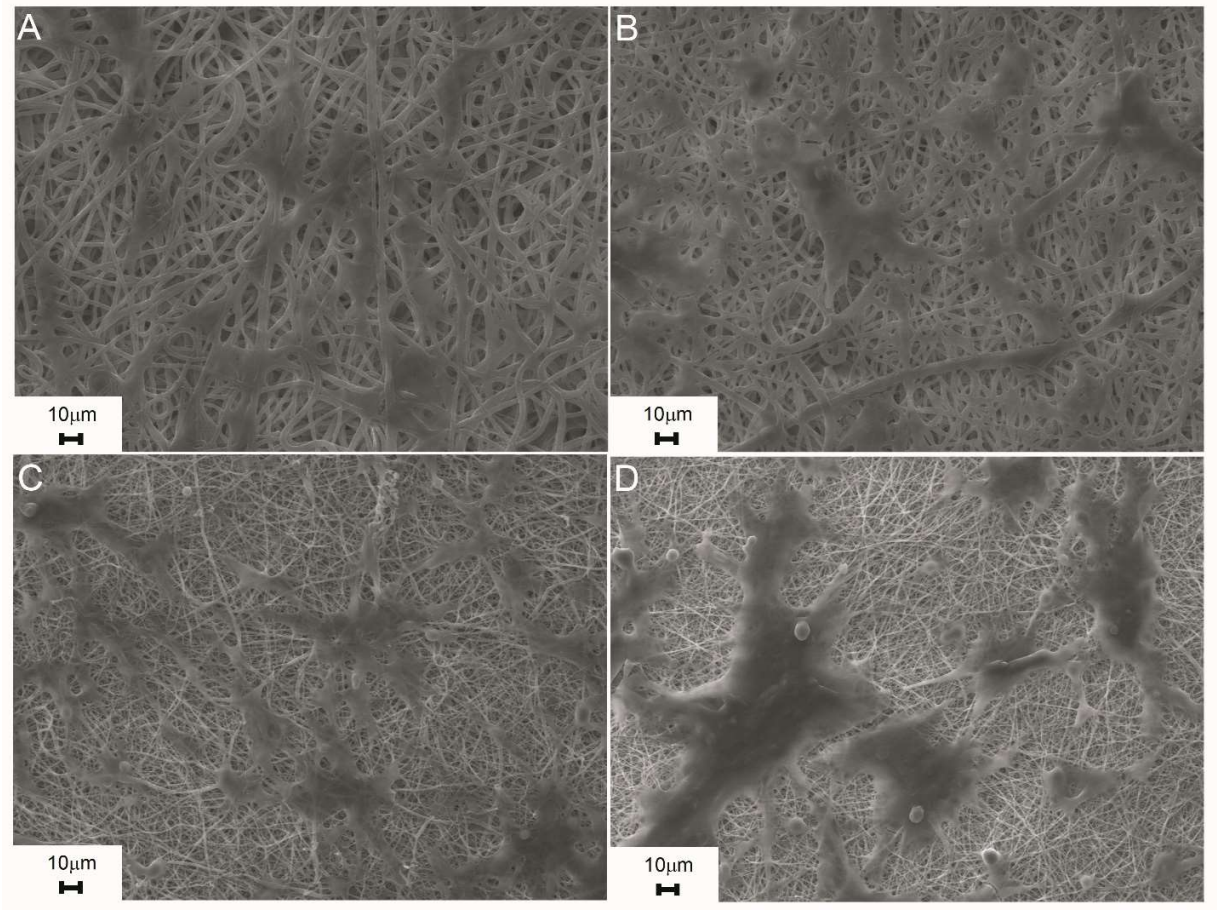

Figure S4. Scanning electron microscopy (SEM) micrographs of fibroblast cells (BHK-21) attached to porous and non-porous electrospun PCL microfiber and nanofiber scaffolds without and with CAM (magnification 1000x). **A.** Porous PCL microfiber scaffold; **B.** Porous PCL microfiber scaffolds with CAM; **C.** Non-porous nanofiber scaffold; **D.** Non-porous nanofiber scaffold with CAM.

Bacterial cell attachment and biofilm formation using *P. aeruginosa* as a model bacterium

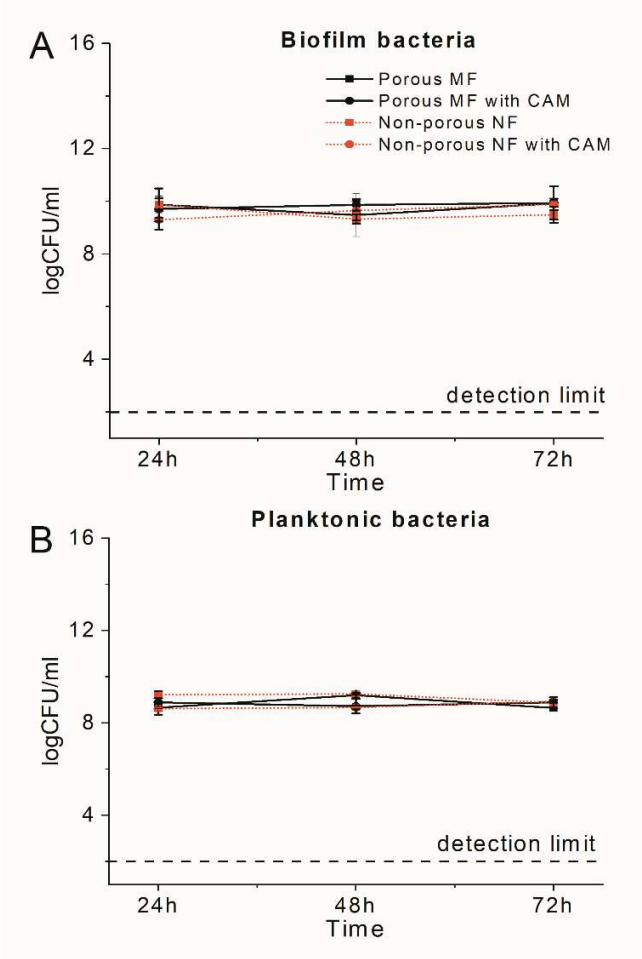

Figure S5. **A.** Antibacterial activity of porous and non-porous electrospun PCL microfiber and nanofiber scaffolds without and with CAM on *P. aeruginosa* **B.** and biofilm formation and antibiofilm activity. Regarding exact formulations reference is made to **Figure 1**. Key: biofilm bacteria – biofilm bacteria washed off from fiber scaffold; CAM – chloramphenicol; MF- microfiber; NF- nanofiber; planktonic bacteria –bacteria from the well where fiber scaffold was removed. Detection limit refers to the CFU that can be counted by plate method (200 CFU/ml).