Supporting Information

Polyhydroxyoctanoate films reinforced with titanium dioxide microfibers for biomedical application

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1. Experimental

1.1. Materials

All chemicals, reagents and cell culture media were purchased from Sigma-Aldrich or Gibco. TiO_2 microfibers (MFs) (polycrystalline anatase-rutil, diameter 400 - 800 nm, length in range from two to hundreds of μm) were supplied by Pardam Nanotechnology Ltd (Roudnice, Czech Republic), **Fig. S1**. Medium chain polyhydroxyalkanoate used for the composites fabrication is a homopolymer poly(3-hydroxyoctanoate), PHO, contained >95% 3-hydroxyoctanoic acid as the monomer [1], was supplied by Bioplastech Ltd (Dublin, Ireland). The number-average (Mn) and weight-average (Mw) molecular weight of the biopolymer were 73.0 and 137.0 kDa, respectively, with a polydispersity index (PDI) of 1.88.

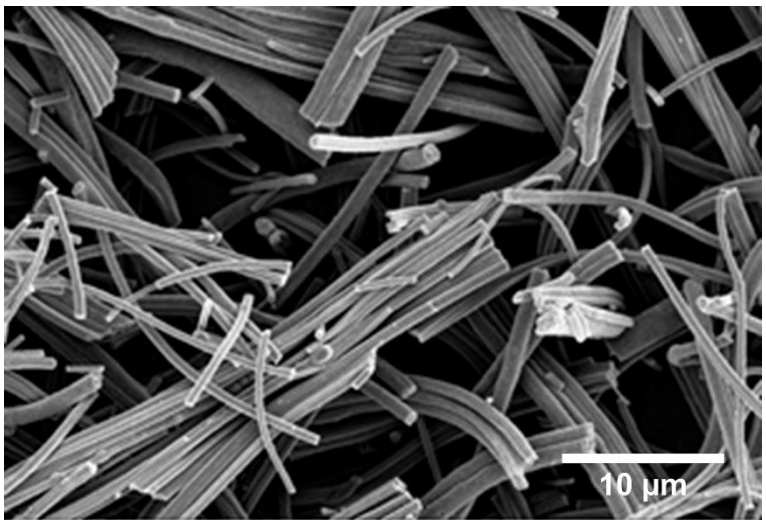

Figure S1. SEM micrograph of the TiO_2 microfibers.

1.2. Characterization

1.2.1. Scanning electron microscopy

Composite surface and internal structure were examined by a high resolution field emission Zeiss Ultra Plus-SEM (Carl Zesis AG, Oberkochen, Germany) at a voltage of 3 kV. For the cross-sections, the samples were cryo-fractured using liquid nitrogen. Before imaging, all samples were sputter coated with a conducting layer of gold-palladium (80/20 ratio).

1.2.2. X-ray diffraction (XRD)

XRD analysis was performed using an X'Pert powder X-ray diffractometer from Malvern Panalytical (Amelo, Nederlands) in Bragg-Brentano geometry. The measurements were performed in the 2θ range of 2 - 80^o with a scan speed of 0.02 ^{o}/s at room temperature. Each sample was measured for 12 h.

To determine the degree of crystallinity, the amorphous phase of the biopolymer in the neat and the composite samples was obtained by melting and subsequent quenching of the samples in liquid nitrogen. The degree of crystallinity (χ_c) was calculated using the following equation (1):

$$\chi_c = \frac{Int_i - Int_0}{Int_i} \times 100\ \%$$

(1)

where Int_i and Int_0 are integrated intensities of the measured patterns of solvent-cast and melt-quenched (amorphous) samples, respectively. The biopolymer crystalline domain size and degree of crystallinity of the biopolymer in the neat and the composite samples were obtained by Rietveld refining the experimental data using the software TOPAS [2,3].

1.2.3. Fourier transform infrared spectroscopy (FTIR)

FTIR spectra were recorded on a Thermo Scientific Nicolet 6700 FT-IR spectrometer using the attenuated total reflectance (ATR) technique from the Smart accessory with diamond crystal (Smart Orbit, Thermo Scientific, Madison, WI, USA). Spectral data were collected in the mid-IR range (4000–500 cm^{-1}) with 32 scans and 4 cm^{-1} resolutions. A background spectrum (16 scans) was recorded before measuring each sample spectrum. Data processing was done using the OMNIC 7 (Thermo Scientific, Madison, WI, USA) software.

1.2.4. Mechanical characterization

Mechanical properties in terms of tensile strength (TS), Young's modulus (YM) and elongation at break (ε) were determined using a ZwickRoell Z005 instrument (Germany) according to the standard of ASTM D 638, with a stretching rate of 50 mm/min^{-1} at room temperature. To ensure the accuracy, each sample was measured five times and the obtained results were averaged.

1.2.5. Cytotoxicity and cellular migration study

Cytotoxicity evaluation was performed according to previously described protocol [4] using human lung fibroblasts MRC-5 cells. In brief, monolayer cell cultures were treated with 50%, 25% and 12.5% (v/v) of filtered biomaterial extract and incubated for 48 h. Cell proliferation was determined using standard MTT assay. Cellular migration study on different substrates was conducted using mouse embryonic fibroblasts MEF 3T3 cell line and procedure described by Witko et al. [5]. In brief, measurements and microscopic observations were made in fluorescent and bright field techniques using a Zeiss Axio Observer Z.1 fluorescent microscope. Image collection, processing and analysis was

performed with Zeiss ZEN Black version 8,1,0,484,1, PALMRobo version V 4.6.0.4 and FluoRender 2.21.0 softwares. For 3D reconstructions a Z-Stack mode was used.

1.3. Statistical analysis

Statistical analysis was done using Student's t-test and one-way ANOVA. The results are presented as mean ± standard deviations (SD). The difference was considered to be statistically significant at $p \leq 0.05$.

2. Results and discussion

2.1. FTIR

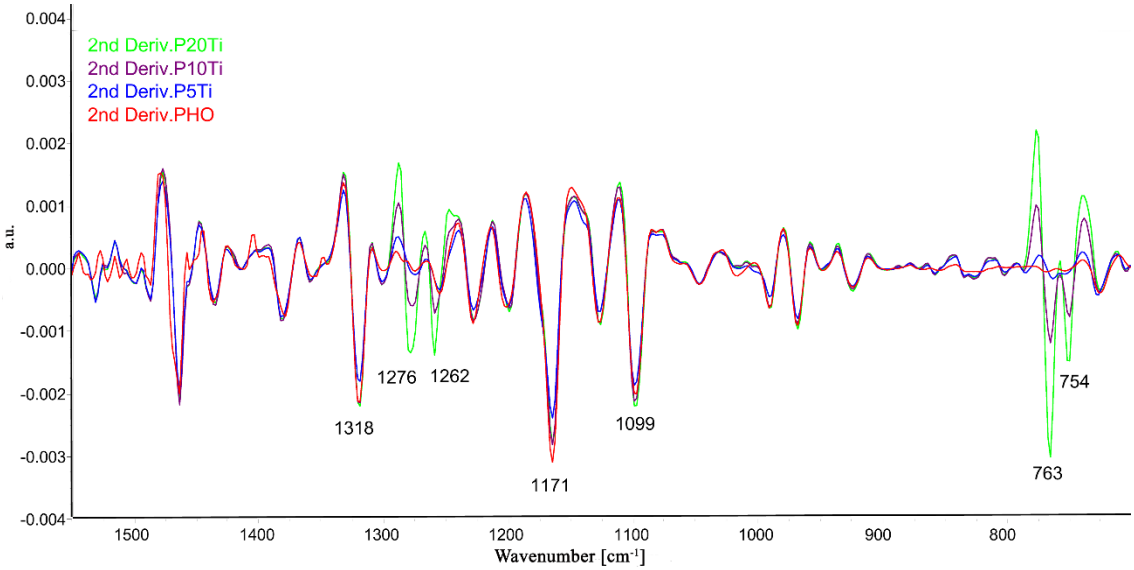

Figure S2. 2nd derivative in the 1500-700 cm^{-1} region of the PHO and PHO/TiO_2 FTIR spectra.

References

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