Supplementary Information (SI)

Biohybrid Electrospun Membrane for the Filtration of Ketoprofen Drug from Water

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SI content:

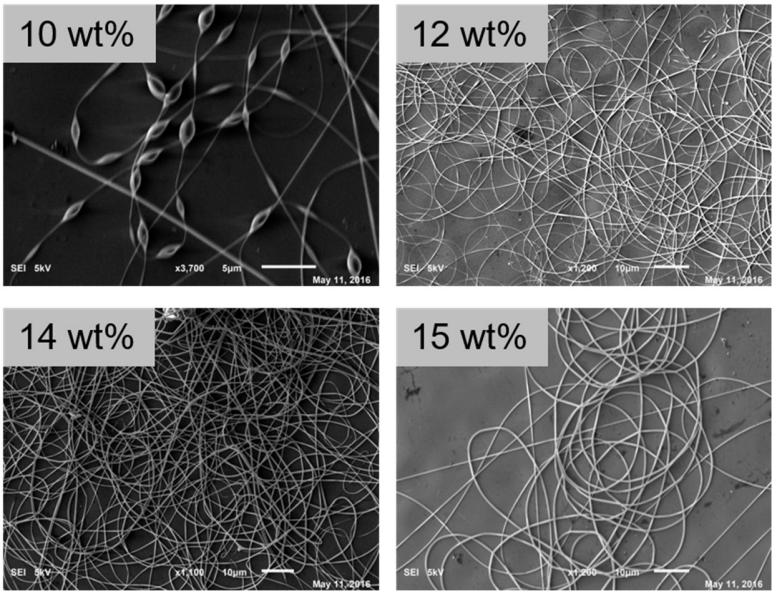

Figure S1. Scanning electron microscope images (SEM) and diameter analysis statistics obtained on 100 measures for each sample of fully hydrolyzed PVA (Mw = 89000-98000) without additives from water. i) 10 wt % PVA water solution gives strongly beaded fibers. ii) 12 wt% of PVA provides nearly uniform fibers with a very low number of beads with 284 ± 52 nm mean diameter. iii) 14 wt% PVA water solution gives a highly uniform electrospun mat with 481 ± 80 nm mean diameter. c) 15 wt% PVA water solution results in highly uniform electrospun fibers with a thicker diameter of 634 ± 86 nm.

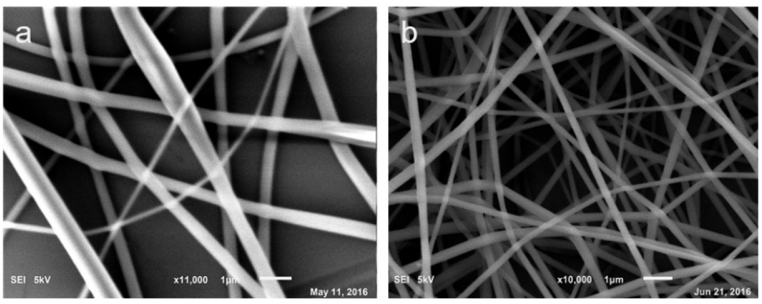

Figure S2. Scanning electron microscope images (SEM) of electrospun mat with different BSA loading (scale bar 1 µm). a) sample from PVA 14 wt% - 0 % BSA loading. Mean diameter 481 ± 80 nm. b) sample from PVA 14 wt% - 10 wt% BSA loading. Mean diameter 201 ± 32 nm.

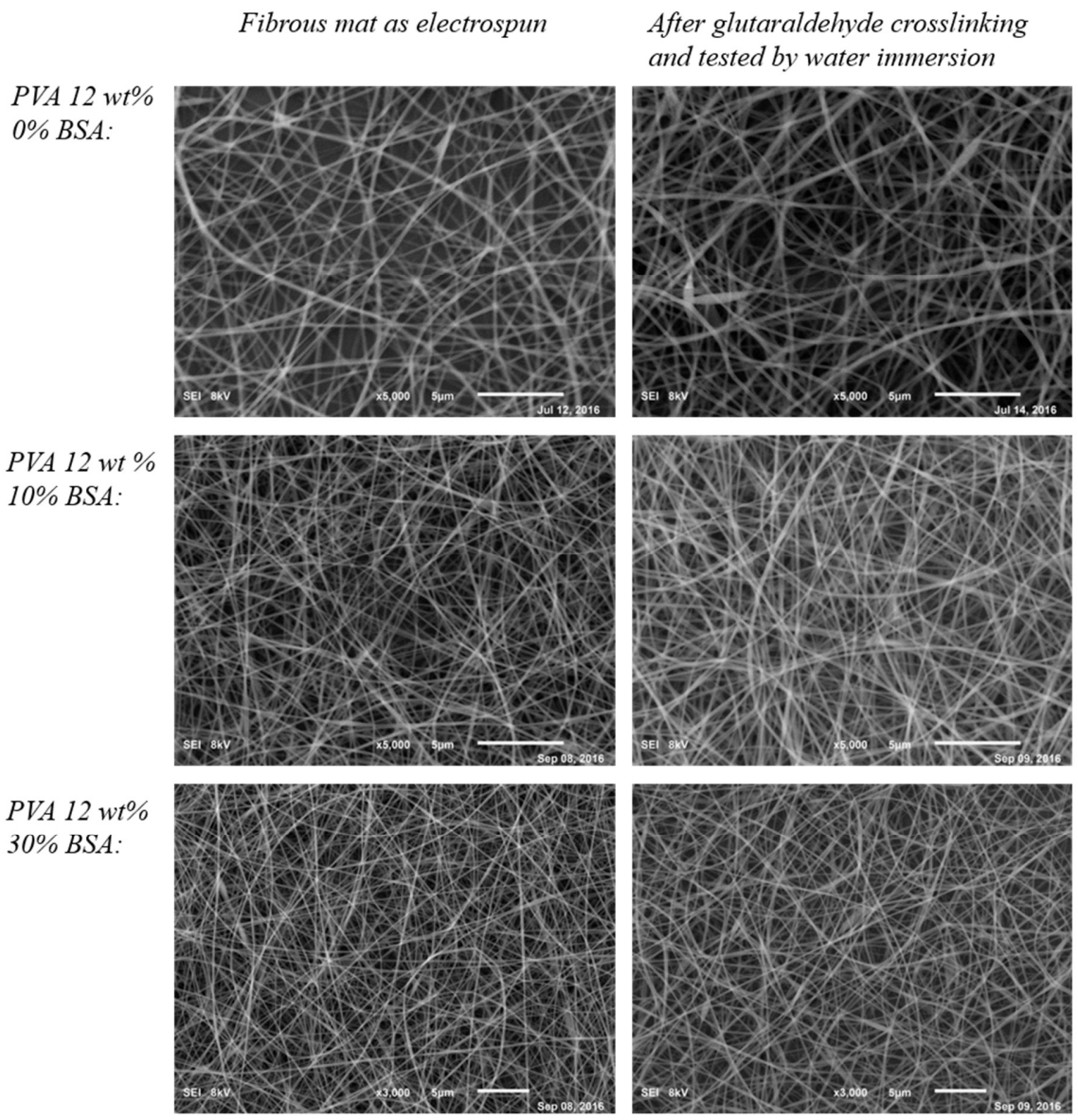

Figure S3. **Chemical crosslinking with Glutaraldehyde (GA):** Scanning electron microscope images (SEM) of electrospun samples with different amount of BSA content, before and after the cross-linking step with glutaraldehyde (GA) and subsequent immersion in water (white scale bar 5 µm). Samples were collected onto a silicon substrate during electrospinning and no further metallization was needed to collect SEM images. On the left: Electrospun mats of 12 wt% PVA with 0 wt%, 10 wt% and 30 wt% BSA content as electrospun. On the right: Electrospun mats of 12 wt% PVA with 0 wt%, 10 wt% and 30 wt% BSA content after cross-linking by immersion in a bath of 0.15 M GA and 0.05 M HCl for 1h and subsequent immersion in water for 3 h to test resistance toward water dissolution.

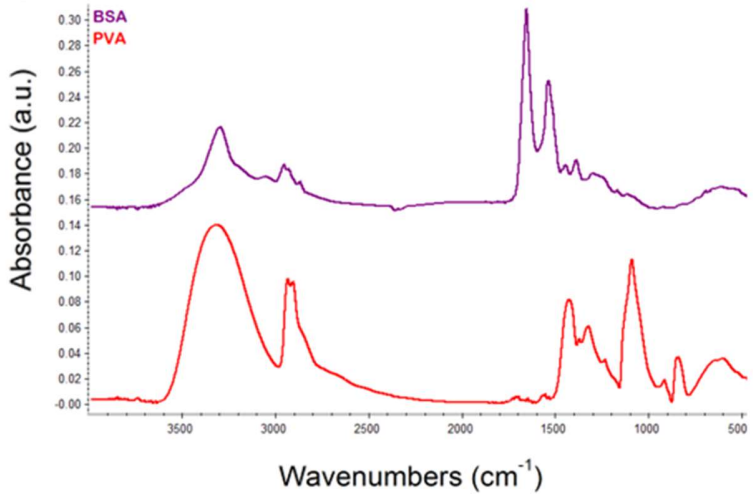

Figure S4. PVA electrospun fibers (in red) and BSA (in purple) FTIR spectra, intensities were offset to better visualize the differences and similarities between the two spectra.

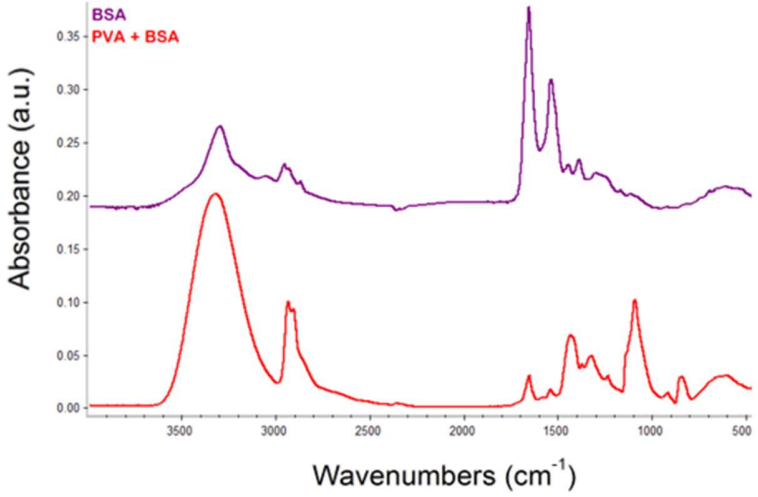

Figure S5. BSA (in purple) and PVA + BSA electrospun fibers (in red) FTIR spectra, intensities were offset to better visualize the differences and similarities between the two spectra.

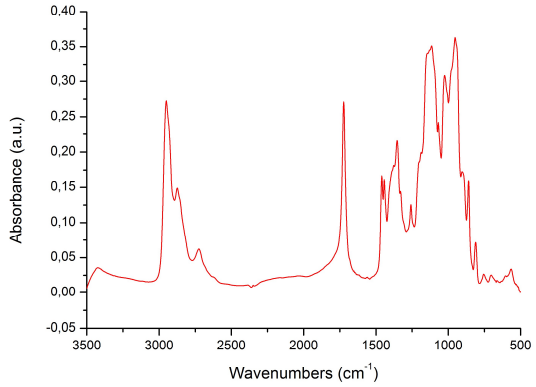

Figure S6. FTIR spectra of the crosslinking solution residuals after nanofibers treatment. The characteristics bands of glutaraldehyde are present while nor amide I nor amide II of BSA are present as a residue.

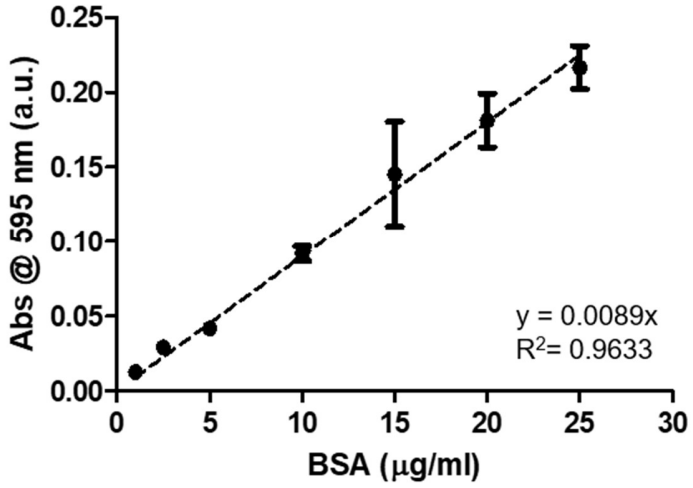

Figure S7 Calibration curves for direct Bradford assay prepared with Coomassie Brilliant Blue G-250 (CBB) and BSA as reference protein. Calibration curve for the direct assay measuring the increasing of absorption at 595 nm with increasing amounts of BSA. Solutions of known BSA concentration were prepared in water; 250 µl of such solutions were mixed with 1.25 ml of CBB and incubated for 5 minutes. Then, absorption at 595 nm was measured using CBB as baseline.

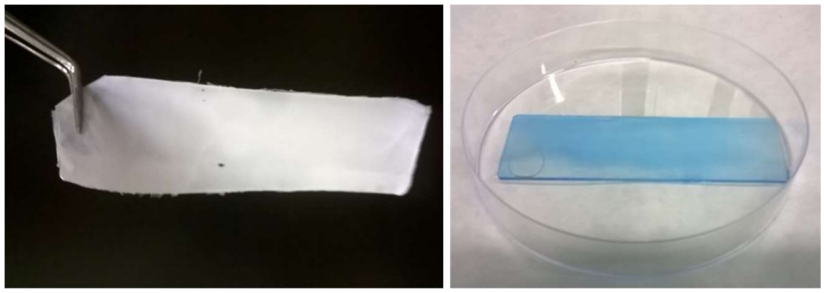

Figure S8 On the left: a self-standing nanofibrous mat electrospun from a solution containing 12 wt% PVA and 10% BSA loading. On the right: an electrospun membrane of the same composition after immersion in a Coomassie Brilliant Blue solution.

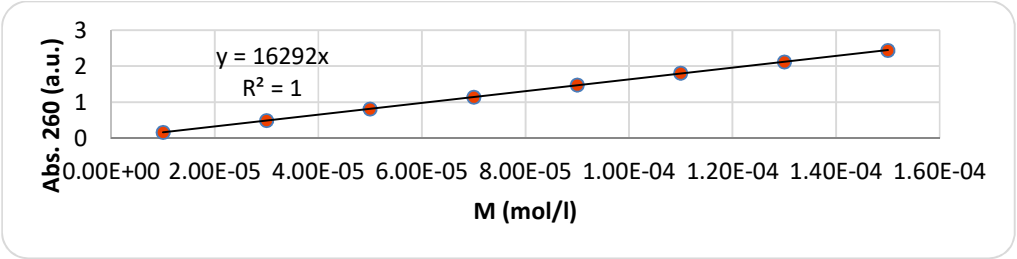

Figure S9 Molar extinction coefficient of ketoprofen in water.

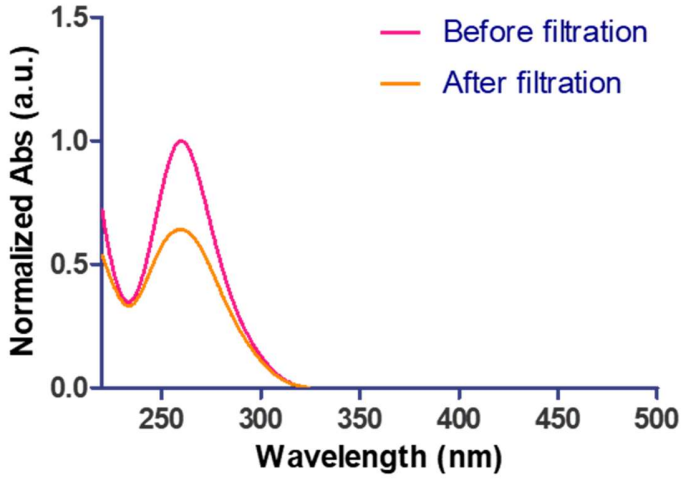

Figure S10 UV-vis absorption spectra before and after filtration of a 2ml ketoprofen water solution [26 µM]. UV-vis absorption spectra before (red spectra) and after passing through the PVA – 10% BSA biohybrid filter (orange spectra). About 40% of the ketoprofen present in solution is retained by the membrane. To assess filter reusability, a gentle washing of the filter by immersion in a bath of distilled water for 30 min at room temperature was performed prior to repeat the filtration experiment. The amount of ketoprofen filtered after washing was less than 40% compared to the first filtration.

Crystal	**BSA-ketoprofen**
PDB code	**6QS9**
Data collection	
Space group	C 2
Cell dimensions	
a (Å)	212.49
b (Å)	44.37
c (Å)	142.71
α (°)	90
β (°)	113.24
γ(°)	90
Wavelength (Å)	1
Resolution (Å)	46.59 – 2.80
R_{sym} *or* R_{merge} *(%)*	3.1 (31.8)
$I/\sigma I$	17.2 (3.3)
Completeness (%)	99.5 (99.5)
Multiplicity	2.8 (2.8)
Refinement	
Resolution (Å)	46.59 – 2.80
No. of reflections	30574
R_{work}/R_{free} *(%)*	19.4/26.2
No. of atoms	
Protein	9230
Ligand	42
Water	80
Average B-factors (Å^2)	93.50
Protein	93.70
Ligand	89.40
Water	72.60
r-m-s-d	
Bond lengths (Å)	0.011
Bond angles (°)	1.78
Ramachandran	
Most favoured (%)	93.00
Additional allowed (%)	6.56
Disallowed (%)	0.44

Table S1. *X-ray data collection and refinement statistics of the BSA-ketoprofen complex crystal structure.*