Supplementary Materials: Antimicrobial Activity of Poly(ester urea) Electrospun Fibers Loaded with Bacteriophages

Angélica Díaz [1], Luis J. del Valle [1], Noel Rodrigo [1], María Teresa Casas [1], George Chumburidze [2], Ramaz Katsarava [2,3,*] and Jordi Puiggalí [1,4,*]

[1] Departament d'Enginyeria Química, EEBE, Universitat Politècnica de Catalunya, Edifici I.2, C/Eduard Maristany, 10-14, 08019 Barcelona, Spain; angelicadiaz07@gmail.com (A.D.); luis.javier.del.valle@upc.edu (L.J.d.V.); nr.arcay@gmail.com (N.R.); m.teresa.casas@upc.edu (M.T.C.)
[2] Georgian Technical University, 77 Kostava str., Tbilisi 0175, Georgia; newagelab.ge@gmail.com
[3] Institute of Chemistry and Molecular Engineering, Agricultural University of Georgia, # 240 David Aghmashenebeli Alley, Tblisi 0159, Georgia
[4] Research Center for Multiscale Science and Engineering, Universitat Politècnica de Catalunya, C/Eduard Maristany, 10-14, 08019 Barcelona, Spain
* Correspondence: r.katsarava@agruni.edu.ge (R.K.); Jordi.Puiggali@upc.edu (J.P.); Tel.: +34-93-401-5649 (J.P.)

Synthesis and characterization of poly(ester urea) 1L6

Poly(ester urea) 1L6 was synthesized with an 91–93% yield, following the synthesis procedure previously reported by Gomurashvili *et al.* [1] and schematized in Figure S1. The synthesis involved two steps: (a) The preparation of the di-*p*-toluenesulfonic acid salt of bis-L-leucinehexane-1,6-diester (L6) by direct condensation of L-leucine with 1,6-hexanediol in refluxing benzene and in the presence of a slight excess of *p*-toluenesulfonic acid (TsOH); (b) Interfacial polycondensation using triphosgene and L6 as starting monomers. Weight and number average molecular weights determined by GPC were 70,000 and 50,000 g/mol, respectively.

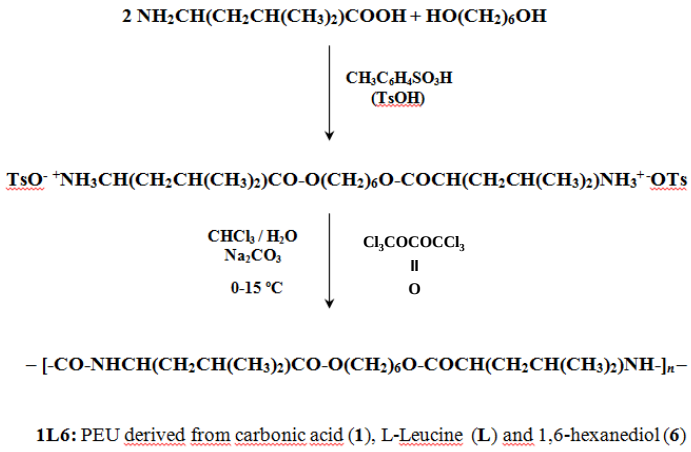

Figure S1. Synthesis scheme of poly(ester urea) 1L6.

Infrared absorption spectrum of poly(ester urea) 1L6 was recorded with a FTIR 4100 Fourier Transform spectrometer from Jasco. A MKII Golden Gate attenuated total reflection (ATR) accessory from Specac was employed. The spectrum showed the typical bands associated to CH_2 (2955 and 2871 cm^{-1}), hydrogen bonded NH (3339 and 1558 cm^{-1}), C=O ester (1734 cm^{-1}), C=O urea (1633 cm^{-1}) and C–O ester (1243 and 1183 cm^{-1}) groups (Figure S2).

X-Ray powder diffraction patterns were obtained with a PANalytical X'Pert PRO MPD θ/θ powder diffractometer with Cu Kα radiation (λ = 1.5418 Å) and a silicon monocrystal sample holder. Operating voltage and current were 40 kV and 50 mA, respectively. Thin samples sandwiched between low absorbing films were used. The X-Ray powder diffractogram of the poly(ester urea) 1L6 sample revealed a semicrystalline character with characteristic reflections at 1.06 and 0.46 nm (Figure S2). By contrast the sample recovered after the electrospinning process became amorphous.

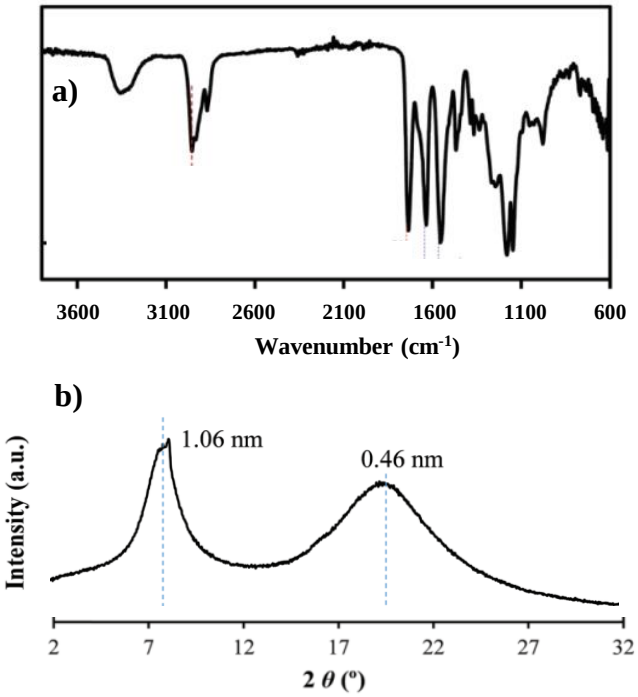

Figure S2. FTIR spectrum (**a**) and X-ray diffraction profile (**b**) of poly(ester urea) 1L6.

Enzymatic degradation of films and scaffolds of poly(ester urea) 1L6

An extensive study on the degradability of both 1L6 films and electrospun scaffolds has recently been published [2]. Weight loss measurements taken during exposure to proteinase K, α-chymotrypsin and lipase media (Figure S3) demonstrated the susceptibility of the 1L6 films to the enzymatic attack. Specifically, weight losses higher than 50% were determined after only 15 days of exposure to all the indicated media. SEM micrographs revealed the clear erosion of film surfaces that contrasted with the practically smooth surfaces observed after the exposure of films into a phosphate buffer saline (PBS) medium (Figure S4 for control and a representative enzymatic medium).

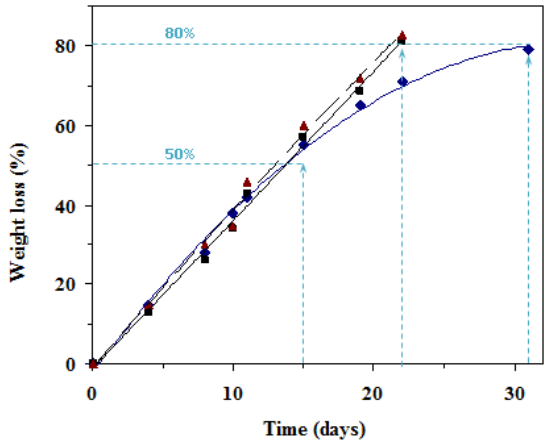

Figure S3. Plots of weight loss versus exposure time for 1L6 films exposed to proteinase K (black symbol), α-chymotrypsin (garnet symbol) and lipase (blue symbol) media. Reproduced with permission from [2], copyright 2015 Elsevier.

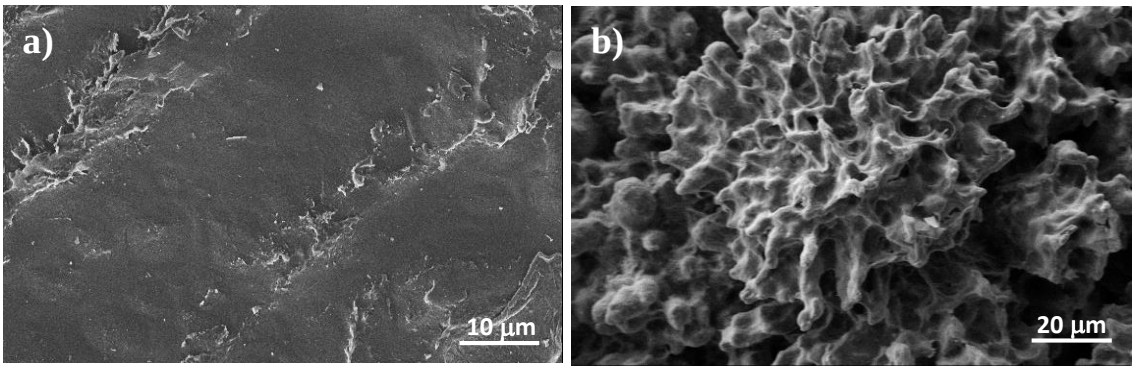

Figure S4. Scanning electron micrographs of 1L6 films after 19 days of exposure to PBS (**a**) and α-chymotrypsin (**b**) media at 37 °C. Reproduced with permission from [2], copyright 2015 Elsevier.

Enzymatic degradation of 1L6 was logically faster when processed as electrospun microfibers. Thus, the weight loss of electrospun scaffolds was higher that 60% after only three days of exposure (Figure S5), being differences between the different enzymatic media not remarkable.

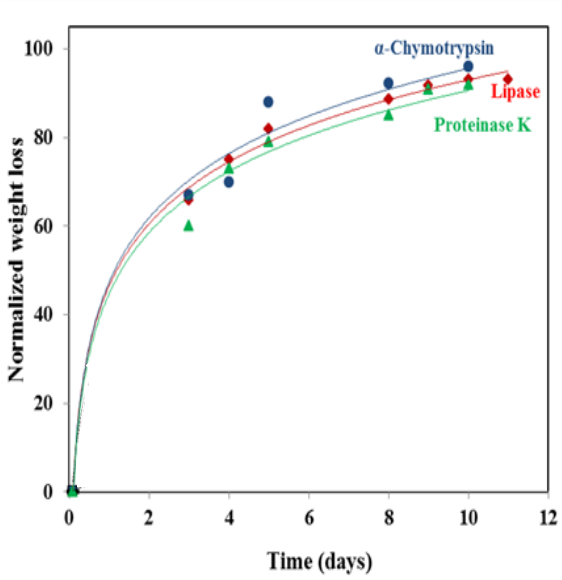

Figure S5. Plots of weight loss versus exposure time for 1L6 scaffolds exposed to proteinase K (green symbols) α-chymotrypsin (blue symbols) and lipase (red symbols) media. Reproduced with permission from [2], copyright 2015 Elsevier.

SEM micrographs revealed that the outer fibers of electrospun scaffolds were logically degraded at the beginning of the exposure to the enzymatic media (Figure S6 for a representative α-chymotrypsin medium), being the inner parts more protected due to the high hydrophobicity of the sample. At longer exposure times the internal fibers became logically accessible to the enzymes due to the advanced degradation of the outer fibers. A complete degradation could be achieved after approximately 10 days of exposure.

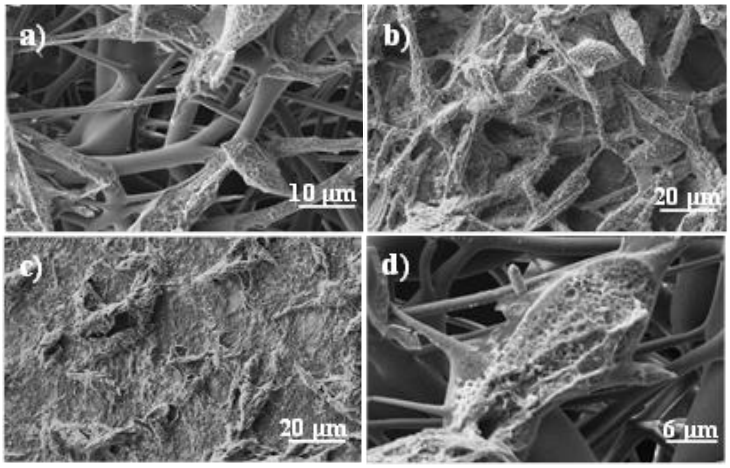

Figure S6. SEM micrographs of 1L6 scaffolds after exposure to an α-chymotrypsin medium at 37 °C for 3 (**a**,**d**), 6 (**b**) and 8 (**c**) days. Reproduced with permission from [2], copyright 2015 Elsevier.

Effect of the electrical field on the morphology and activity of phages

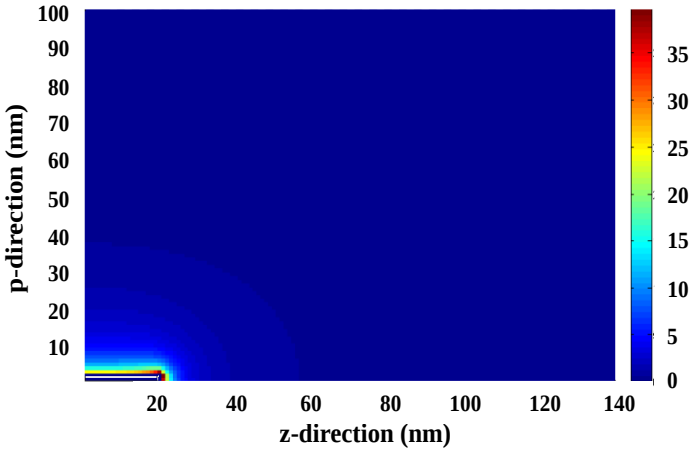

Figure S7. Calculated modulus of the electric field for a potential of 30 kV.

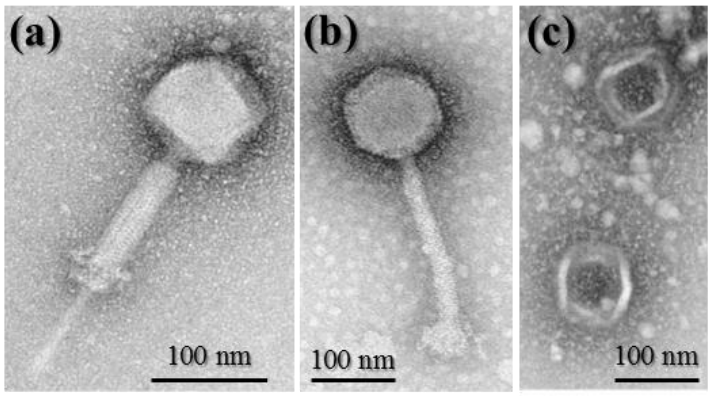

Figure S8. TEM micrographs showing the morphology of *Myoviridae* (**a**), *Siphoviridae* (**b**) and *Leviviridae* (c) phages that form part of the Phagestaph commercial preparation after exposition to a 40 kV/cm electric field. Scale bars 100 nm.

Electrospinning of PEU 1L6 loaded with bacteriophages

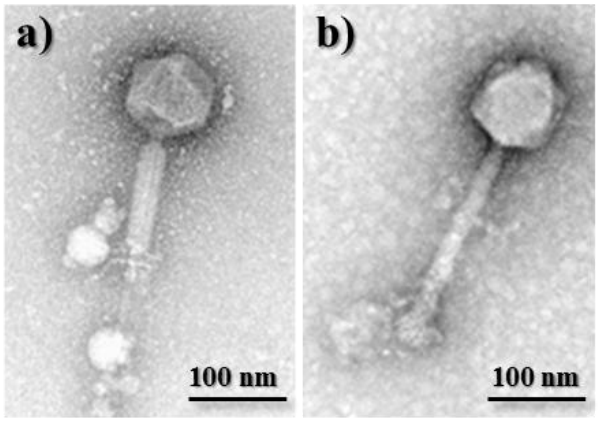

Figure S9. TEM micrographs showing the morphology of representative *Siphoviridae* (**a**) and *Leviviridae* (**b**) phages that were recovered from electrospun 1L6 scaffolds directly loaded with phages (**a**) and with carbonate particles having adsorbed phages (**b**).

References

1. Gomurashvili, Z.D.; Katsarava, R.; Tugushi, D. Poly(ester urea) polymers and methods of use, WO 2007050415 A2, 3 May 2007.
2. Díaz, A.; del Valle, L.J.; Tugushi, D.; Katsarava, R.; Puiggalí, J. New poly(ester urea) derived from L-leucine: Electrospun scaffolds loaded with antibacterial drugs and enzymes. *Mater. Sci. Eng. C,* **2015**, *46*, 450-462.