Large three-dimensional poly(glycerol sebacate)-based scaffolds - A freezedrying preparation approach

Martin Frydrych and Biqiong Chen*

Department of Materials Science and Engineering, University of Sheffield, Mappin Street, Sheffield, S1 3JD, UK.

*To whom correspondence should be addressed. E-mail: <u>biqiong.chen@sheffield.ac.uk</u> Tel: +44 (0) 114 222 5958 Fax: +44 (0) 114 222 5943

Supporting Information

Fig. S1 presents cracks and holes in the surface of cured poly(glycerol sebacate) (PGS) film, after 24 h ethanol extraction.



Fig. S1: (a1-2) SEM micrographs of the cured PGS film surface, after 24h ethanol extraction ((a1) scale bar: 200µm, (a2) scale bar: 50µm).

Fig. S2 demonstrates pristine poly(L-lactic acid) (PLLA) scaffolds after the freeze-drying process.



Fig. S2: (a1) Top and (a2) side view (cross section) of non-cured freeze-dried PLLA scaffold, produced with 2 g PLLA and 40 mL 1,4-dioxane (scale bar: 1cm).

Fig. S3 presents the effect of the weight ratio of the PGS pre-polymer (pre-PGS) to PLLA on the microstructure of the pre-PGS/PLLA scaffold. A higher weight ratio of PLLA demonstrated a more uniform circular and regular cell structure, while a higher weight ratio of pre-PGS resulted in distracted and non-uniform cell shapes.



Fig. S3:SEM micrographs of pre-PGS/PLLA blend foams with a weight ratios of (a) 2:3, (b) 2:2 and (c) 2:1 (scale bar: 100µm).

Fig. S4-7 demonstrate the final microstructure of PGS/PLLA scaffolds after the curing process.



Fig. S4: SEM micrographs of cured PGS/PLLA blend scaffolds with a weight ratios of (a) 2:2, (b) 2:3 (scale bar: 500 µm).



Fig. S5: SEM micrographs of cured PGS/PLLA blend scaffolds with a weight ratios of (a) 1:2, (b) 2:1 (scale bar: 500 µm).



Fig. S6: SEM micrographs of cured PGS/PLLA blend scaffolds with a weight ratios of (a) 2.5:1, (b) 3:1 (scale bar: $500 \mu m$).



Fig. S7: SEM micrographs of cured PGS/PLLA blend scaffolds with a weight ratios of (a) 3.5:1, (b) 4:1 (scale bar: 500 µm).

Figs. S8 and S9 present the physical shape of cured PGS/PLLA scaffolds with a weight ratios of 2.5:1 and 3:1, respectively, indicating homogenous structures as well as no physical changes after the curing stage.



Fig. S8: (a1) Top and (a2) side view of cured PGS/PLLA blend scaffold with a weight ratios of 2.5:1 (scale bar: 1cm).



Fig. S9: (a1) Top and (a2) side view of cured PGS/PLLA blend scaffold with a weight ratios of 3:1 (scale bar: 1cm).

Figs. S10 and S11 illustrate cured PGS/PLLA scaffolds with weight ratios of 3.5:1 and 4.1, respectively, presenting the evidence of structure collapse.



Fig. S10: (a1) Top and (a2) side view of cured PGS/PLLA blend scaffold with a weight ratio of 3.5:1 (scale bar: 1cm).



Fig. S11: (a1) Top and (a2) side view of cured PGS/PLLA blend scaffold with a weight ratio of 4:1 (scale bar: 1cm).

In order to eliminate the effect of scaffold density or porosity, the specific Young's modulus (E_f / ρ_f) and specific tensile strength (σ_{fmax} / ρ_f) of all scaffolds were calculated, as presented in Fig. S12. Cured PGS/PLLA scaffolds with a higher PGS weight ratio showed overall lower specific Young's modulus and lower specific tensile strength values (with the exception of the two collapsed scaffolds), with the lowest specific Young's modulus and specific tensile strength values of 2.02 MPa (Mg m⁻³)⁻¹ and 0.17 MPa (Mg m⁻³)⁻¹ found for the PGS/PLLA scaffold with a weight ratio of 3:1. This represented a decrease of the specific Young's modulus and specific tensile strength of 96% and 92%, respectively, compared to the pure PLLA scaffold. Both scaffolds only had a difference of 28% in cell size, implying that the reductions in the specific modulus and specific tensile strength can be attributed primarily to the high PGS content. Hence, higher PGS ratios result into soft and elastomeric scaffolds.



Fig. S12: Specific Young's modulus and specific tensile strength for PLLA and cured PGS/PLLA blend scaffolds with different weight ratios.

Fig. S13 presents cured PGS film samples before and during the in vitro degradation tests in enzyme-free and enzyme-containing PBS solution.



Fig. S13: Pictures of PGS (cured at 150 °C for 24 h) film samples during incubation in enzymefree PBS solution ((a1): Day 0; (a2): Day 1; (a3): Day 7, (a4): Day 15; (a5): Day 23; (a6): Day: 31), and in PBS solution with the addition of lipase enzyme ((b1): Day 0; (b2): Day 1; (b3): Day 7, (b4): Day 15; (b5): Day 23; (b6): Day: 31) under dynamic conditions for up to 31 days at 37 °C (scale bar: 1 cm).

Fig. S14 presents SEM micrographs of the PGS film samples after the completion of in vitro degradation tests in enzyme-free and enzyme-containing PBS solution.



Fig. S14: SEM micrographs of PGS film (cured at 150 °C for 24 h) specimens with a weight ratio of 2.5:1, after 31 days at 37 °C in (a1-3) enzyme-free PBS solution and (b1-3) in enzyme-containing PBS solution ((a1, b1) scale bar: 1 cm; (a2, b2) scale bar: 100 μ m; (a3, b3) scale bar: 30 μ m).

Fig. S15 presents PLLA scaffolds samples before and during the in vitro degradation tests in enzyme-free and enzyme-containing PBS solution.



Fig. S15: Pictures of PLLA scaffold samples during incubation in PBS solution ((a1): Day 0; (a2): Day 1; (a3): Day 7, (a4): Day 15; (a5): Day 23; (a6): Day: 31), and in PBS solution with the addition of lipase enzyme ((b1): Day 0; (b2): Day 1; (b3): Day 7, (b4): Day 15; (b5): Day 23; (b6): Day: 31) under dynamic conditions for up to 31 days at 37 °C (scale bar: 1 cm).

Fig. S16 presents SEM micrographs of the PLLA scaffold samples after the completion of in vitro degradation tests in enzyme-free and enzyme-containing PBS solution.



Fig. S16: SEM micrographs of PLLA scaffold specimens, after 31 days at 37 $^{\circ}$ C in (a1-3) enzyme-free PBS solution and (b1-3) in enzyme-containing PBS solution ((a1, b1) scale bar: 1 cm; (a2, b2) scale bar: 100 µm; (a3, b3) scale bar: 30 µm).

a1 **b1 S1 S2 S**3 **S2 S1 S**3 a2 b2 **S1 S2 S**3 **S2 S1 S**3 a3 b3 **S1 S2** \$3 **S2 S1 S**3 a4 b4 **S2 S**3 **S1 S1 S2** a5 b5 **S1 S2** \$3 **S1 S2** \$3 a6 b6 **S2 S**3 **S2 S**3 **S1 S1**

Fig. S17 presents PGS/PLLA scaffolds samples with a weight ratio of 2.5:1 before and during the in vitro degradation tests in enzyme-free and enzyme-containing PBS solution.

Fig. S17: Pictures of PGS/PLLA scaffold samples with a weight ratio of 2.5:1 during incubation in PBS solution ((a1): Day 0; (a2): Day 1; (a3): Day 7, (a4): Day 15; (a5): Day 23; (a6): Day: 31), and in PBS solution with the addition of lipase enzyme ((b1): Day 0; (b2): Day 1; (b3): Day 7, (b4): Day 15; (b5): Day 23; (b6): Day: 31) under dynamic conditions for up to 31 days at 37 °C (scale bar: 1 cm).

Fig. S18 presents SEM micrographs of the PGS/PLLA scaffolds samples with a weight ratio of 2.5:1 after the completion of in vitro degradation tests in enzyme-free and enzyme induced PBS solution.



Fig. S18: SEM micrographs of PGS/PLLA scaffold specimens with a weight ratio of 2.5:1, after 31 days at 37 °C in (a1-3) enzyme-free PBS solution and (b1-3) in enzyme-containing PBS solution ((a1, b1) scale bar: 1 cm; (a2, b2) scale bar: 100 μ m; (a3, b3) scale bar: 30 μ m).

Fig. S19 presents PGS/PLLA scaffolds samples with a weight ratio of 3:1 before and during the in vitro degradation tests in enzyme-free and enzyme-containing PBS solution.

Fig. S19: Pictures of PGS/PLLA scaffold samples with a weight ratio of 3:1 during incubation in PBS solution ((a1): Day 0; (a2): Day 1; (a3): Day 7, (a4): Day 15; (a5): Day 23; (a6): Day: 31), and in PBS solution with the addition of lipase enzyme ((b1): Day 0; (b2): Day 1; (b3): Day 7, (b4): Day 15; (b5): Day 23; (b6): Day: 31) under dynamic conditions for up to 31 days at 37 °C (scale bar: 1 cm).

Fig. S20 presents SEM micrographs of the PGS/PLLA scaffolds samples with a weight ratio of 3:1 after the completion of in vitro degradation tests in enzyme-free and enzyme-containing PBS solution.

Fig. S20: SEM micrographs of PGS/PLLA scaffold specimens with a weight ratio of 2.5:1, after 31 days at 37 °C in (a1-3) enzyme-free PBS solution and (b1-3) in enzyme-containing PBS solution ((a1, b1) scale bar: 1 cm; (a2, b2) scale bar: 100 μ m; (a3, b3) scale bar: 30 μ m).