Supporting Information

Functional Nanomaterials and 3D-printable Nanocomposite Hydrogels for Enhanced Cell Proliferation and for the Reduction of Bacterial Biofilm Formation

Andisheh Motealleh,¹ Didem Kart,² Michael Czieborowski,¹ and Nermin S. Kehr^{*1}

¹Dr. N. S. Kehr, Dr. A. Motealleh, and M. Czieborowski Physikalisches Institute and Center for Soft Nanoscience, Westfälische Wilhelms-Universität Münster, Busso-Peus-Strasse 10, 48149 Münster, Germany E-mail: seda@uni.muenster.de

²Dr. Didem Kart

Department of Pharmaceutical Microbiology, Hacettepe University Faculty of Pharmacy, 06100 Sihhiye, Ankara, Turkey

Determination of the swelling ratio of AlgL, AlgL-PMO-OH, AlgL-PMO-PDL, AlgL^{Tet}**PMO-OH, and AlgL**^{-Tet}**PMO-PDL scaffolds:** The weight of dried scaffolds was first measured then they were immersed in cell culture media for 1 day, 4 and 7 days at physiological temperature (37 °C). The swollen scaffolds were removed from cell culture media and weighed again. The swelling ratio (SR) was calculated using the following equation:

$$SR = Ws - Wd/Wd$$

where Ws is the mass of the swollen scaffolds and Wd is their dry mass; all experiments were carried out in triplicate.

Degradation behavior of AlgL, AlgL-PMO-OH, AlgL-PMO-PDL, AlgL-^{Tet}**PMO-OH, and AlgL-**^{Tet}**PMO-PDL scaffolds:** Each scaffold (in the presence and absence of cells) was covered with cell culture media (2 ml) and incubated for 1 day, 4 and 7 days at 37 °C. After each incubation time, the samples were taken out and dried at 37 °C. The degradation behavior [weight loss (%)] was found by measuring the weight of the samples before and after immersing them in cell culture media according to the following equation:

$$W_{loss}$$
 (%) = $[W_1 - W_2/W_1] * 100$

where W_1 and W_2 indicate the weight before and after degradation, respectively.

Rheological measurements: Rheological measurements were done using an MCR 302 rheometer (Anton Paar, Ashland, VA, USA) with a 25 mm diameter parallel-plate geometry measuring system. Storage modulus (G'), loss modulus (G") and complex viscosity ($|\eta^*|$) were measured from an amplitude sweep of AlgL, AlgL-PMO-OH, AlgL-PMO-PDL, AlgL-^{Tet}PMO-OH, and AlgL-^{Tet}PMO-PDL hydrogels in a linear viscoelastic range at a frequency range from 0.01 to 100 Hz. The measured viscosity curves were obtained from the rotational test, which was performed at shear rates ranging from 1 to 10 s⁻¹. In our study, a 25 °C temperature was defined for all experiments.

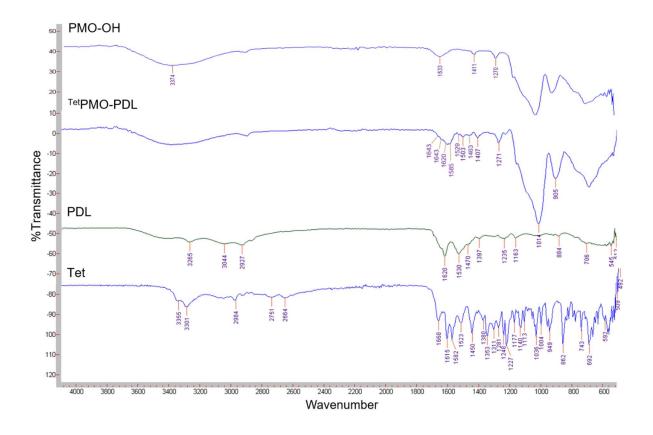


Figure S1. The IR spectra of PMO-OH, ^{Tet}PMO-PDL, PDL, and Tet.

Table S1. The DLS and zeta	potential of PMO-OH.	, PMO-PDL, Tet	^t PMO-OH, and	^{Tet} PMO-PDL.

	РМО-ОН	PMO-PDL	TetPMO-OH	TetPMO-PDL
DLS (nm)	250.9 ± 7.2	610.9 ± 42.3	374.8 ± 33.0	622.1 ± 33.2
Zeta potential (mV)	$\textbf{-25.9}\pm0.4$	33.8 ± 0.4	$\textbf{-26.6} \pm 0.7$	35.0 ± 1.2

Table S2. The amount of PDL and Tet on PMO nanomaterials.

	^{Tet} PMO-OH	TetPMO-PDL	PMO-PDL
PDL (µg/mg)		4.2 ± 0.2	3.9 ± 0.3
Tet (µg/mg)	264.0 ± 15.6	238.6 ± 9.1	

Table S3. Swelling ratio of scaffolds.

	AlgL	AlgL- PMO-OH	AlgL- PMO-PDL	AlgL- ^{Tet} PMO-OH	AlgL- ^{Tet} PMO-PDL
1 d	22.5 ± 2.5	24.3 ± 2.1	26.1 ± 2.5	23.9 ± 1.8	25.8 ± 2.1
4 d	22.8 ± 2.2	24.9 ± 2.4	26.6 ± 2.4	24.1 ± 2.0	26.0 ± 2.1
7 d	22.7 ± 2.1	24.8 ± 2.4	26.5 ± 2.4	24.0 ± 2.0	25.9 ± 2.1

Table S4. Weight loss (%) of scaffolds.

without cells	AlgL	AlgL- PMO-OH	AlgL-PMO- PDL	AlgL- ^{Tet} PMO-OH	AlgL- ^{Tet} PMO-PDL
1d	0.4 ± 0.1	0.2 ± 0.04	0.1 ± 0.04	0.2 ± 0.1	0.1 ± 0.1
4d	2.5 ± 1.1	0.8 ± 0.2	0.5 ± 0.1	0.8 ± 0.1	0.4 ± 0.1
7d	4.0 ± 0.5	1.8 ± 0.6	1.1 ± 0.2	1.7 ± 0.3	1.1 ± 0.3

with cells	Alg-Lap	Alg-Lap- PMO-OH	Alg-Lap- PMO-PDL	Alg-Lap- ^{Tet} PMO-OH	Alg-Lap- ^{Tet} PMO-PDL
1d	8.0 ± 0.9	5.5 ± 1.0	4.9 ± 0.6	5.4 ± 1.2	4.7 ± 1.9
4d	10.0 ± 1.9	7.0 ± 1.1	6.3 ± 0.7	6.7 ± 1.5	6.2 ± 1.4
7d	11.4 ± 2.7	7.4 ± 1.6	6.8 ± 1.6	7.2 ± 0.8	6.6 ± 1.2

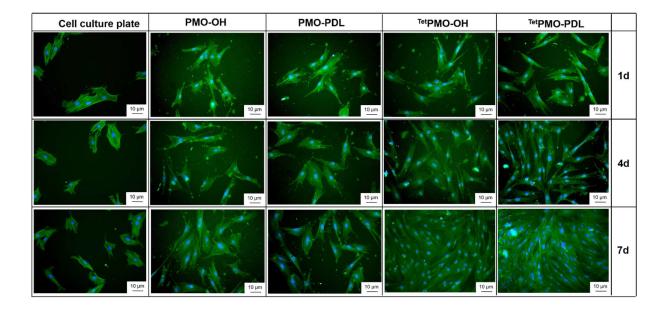


Figure S2. Fluorescence microscopy images (20x magnification) of fibroblast cells in 0.3 mg/ml PMO after 1 day, 4 days and 7 days (Blue: DAPI-stained cell nuclei. Green: Phalloidin-stained cell actin filaments).

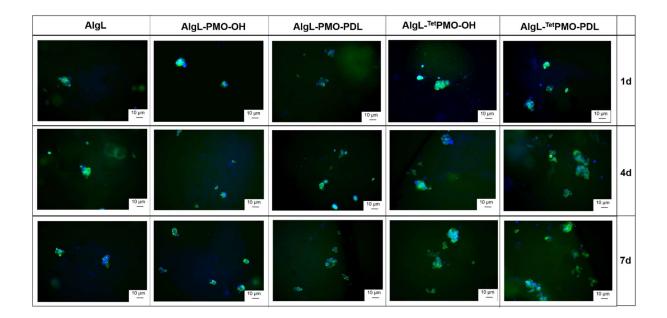


Figure S3. Fluorescence microscopy images (20x magnification) of fibroblast cells in scaffolds after 1 day, 4 days and 7 days (the red line shows the scale bar of $\sim 10 \mu$ m) (Blue: DAPI-stained cell nuclei. Green: Phalloidin-stained cell actin filaments).