

Supporting Information

Injectable Oxygen Generating Nanocomposite Hydrogels with Prolonged
Oxygen Delivery for Enhanced Cell Proliferation under Hypoxic and Normoxic
Conditions

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Pore size and porosity measurement of AlgL, ¹BPO-AlgL and ³BPO-AlgL scaffolds: The ethanol replacement method was used for porosity measurement. Dried nanocomposite hydrogel scaffolds were immersed in absolute ethanol overnight and weighed after blotting the excess ethanol on the surface. The porosity was calculated from the following equation:

$$\text{Porosity (\%)} = ((M2 - M1) / \rho V) * 100 \quad (4)$$

Where, M1 and M2 are the mass of scaffolds before and after the immersion in absolute ethanol, respectively; ρ is the density of absolute ethanol and V is the volume of the samples.

Determination of the swelling ratio of AlgL, ¹BPO-AlgL and ³BPO-AlgL scaffolds: The weight of dried scaffolds was first measured then they were immersed in cell culture media for 1 day 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:

$$\text{SR} = (W_s - W_d) / W_d \quad (1)$$

where W_s is the mass of the swollen scaffolds and W_d is the dry mass of them and all experiments were carried out in triplicate.

Degradation behavior of AlgL, ¹BPO-AlgL and ³BPO-AlgL scaffolds: Each scaffold was covered with cell culture media (2 mL) in the presence (10,000) of fibroblast and Colo 818 cells and incubated for 1 day and 7 days at 37 °C and 5% CO₂. 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:

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

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, 1 BPO-AlgL and 3 BPO-AlgL 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} . The duration of a data point was defined by the instrument automatically from 2 to 90 s. In our study, a 25 °C temperature was defined for all experiments.

Mechanical properties of AlgL, 1 BPO-AlgL and 3 BPO-AlgL scaffolds: Mechanical properties of samples were analyzed using compressive tests. Samples of cylindrical discs (10 mm height and 10 mm diameter) in 3D mold were prepared. Compressive modulus was calculated based on the slope of the compressive stress/strain curve.

Figure S1. The suggested reaction mechanism of the BPO degradation in aqueous solutions.

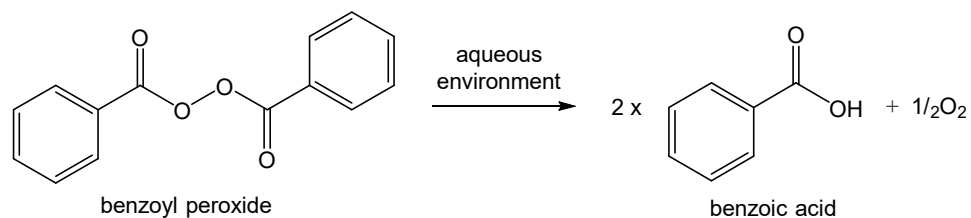


Table S1. Porosity (%) of AlgL, 1 BPO-AlgL, and 3 BPO-AlgL scaffolds.

	AlgL	1 BPO-AlgL	3 BPO-AlgL
Porosity	71.0 ± 6.3	66.2 ± 7.3	63.0 ± 7.3

Table S2. Swelling ratio (%) of AlgL, 1 BPO-AlgL, and 3 BPO-AlgL scaffolds after 1 day and 7 days incubation at 37°C.

	AlgL	¹BPO-AlgL	³BPO-AlgL
1d	60.2 ± 4.3	48.7 ± 2.3	39.9 ± 3.1
7d	59.7 ± 3.0	47.7 ± 3.4	38.8 ± 3.6

Table S3. Degradation (%) of AlgL, ¹BPO-AlgL, and ³BPO-AlgL scaffolds in the presence of cells under hypoxic and normoxic conditions.

Fibroblast cells				
		AlgL	¹BPO-AlgL	³BPO-AlgL
1d	normoxic	3.4 ± 1.0	1.0 ± 0.2	0.7 ± 0.1
7d	normoxic	4.5 ± 1.1	2.5 ± 0.5	1.0 ± 0.2
1d	hypoxic	2.8 ± 1.1	0.7 ± 0.1	0.4 ± 0.1
7d	hypoxic	3.2 ± 1.5	1.4 ± 0.1	0.8 ± 0.3

Colo 818 cells				
		AlgL	¹BPO-AlgL	³BPO-AlgL
1d	normoxic	4.1 ± 0.6	2.1 ± 0.2	1.3 ± 0.2
7d	normoxic	5.3 ± 1.1	3.2 ± 0.5	1.7 ± 0.4
1d	hypoxic	3.5 ± 0.7	1.2 ± 0.6	0.8 ± 0.3
7d	hypoxic	4.1 ± 0.5	2.2 ± 0.7	1.1 ± 0.5