Supporting Information

Directed vertical cell migration via bifunctionalized nanomaterials in 3D step-gradient nanocomposite hydrogels

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General procedure for synthesis of ^{Dex}PMO-PDL: PMO-OH (200 mg) was suspended in 1 ml ethanol and then mixed with Dex (20 mg) dissolved in 1 ml ethanol. This reaction was stirred for 1 day at room temperature. The final product ^{Dex}PMO-OH was achieved by centrifugation and dried at room temperature. To functionalize the ^{Dex}PMO-OH particles with PDL, these particles were suspended with a PDL solution (0.5 mg/ml) and sonicated for 10 min. This reaction mixture was stirred overnight at room temperature. The final products ^{Dex}PMO-PDL were achieved by centrifugation and dried at room temperature.

Dex release kinetics from $^{0.3}$PMO-Alg, and $^{0.9}$PMO-Alg scaffolds: UV spectrophotometry was used to quantitatively determine the released amount of Dex from $^{0.3}$PMO-Alg and $^{0.9}$PMO-Alg scaffolds. First, we prepared the Dex calibration curve using different concentrations of Dex in ethanol. Then the absorption maxima at 242 nm were plotted as a function of the Dex concentration, and the respective slope was evaluated. The relationship $y = 0.172x - 0.02$ was achieved from the plotted graph. Then, to assess the amount of Dex released from $^{0.3}$PMO-Alg and $^{0.9}$PMO-Alg scaffolds, these scaffolds were immersed into 1ml PBS at pH 7.4 then they were kept on a stirrer for 3 min to 7 days. The absorption of the PBS solution after each specific time was measured. The amount of released Dex from $^{0.3}$PMO-Alg and $^{0.9}$PMO-Alg scaffolds was determined using the formula obtained from the Dex calibration curve.

Determination of swelling ratio of ^{0}PMO-Alg, $^{0.3}$PMO-Alg, and $^{0.9}$PMO-Alg scaffolds: The weight of dried ^{0}PMO-Alg, $^{0.3}$PMO-Alg and $^{0.9}$PMO-Alg scaffolds was first measured, then the scaffolds were immersed in cell culture media for 1, 4 and 7 days at physiological temperature (37°C). The swollen scaffolds were taken from cell culture media and weighed again. The swelling ratio (SR) was calculated using the following equation:

$$SR = W_s - W_d / W_d$$

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

Degradation behaviour of ^{0}PMO-Alg, $^{0.3}$PMO-Alg, and $^{0.9}$PMO-Alg scaffolds: The ^{0}PMO-Alg, $^{0.3}$PMO-Alg and $^{0.9}$PMO-Alg scaffolds in the presence and absence of cells were covered with cell culture media (2 ml) and incubated for 1 day, 4 days and 7 days at 37°C and 5% CO_2. After each incubation time, the 3D scaffolds were taken out and dried again at 37°C. The degradation behaviour [weight loss (%)] was determined by measuring the weight of the samples before and after immersing them in cell culture media by following the equation:

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

where W_1 and W_2 are the weights before and after degradation, respectively. The results reported are averages and standard deviations of three measurements.

Pore size and porosity measurement of ^{0}PMO-Alg, $^{0.3}$PMO-Alg, and $^{0.9}$PMO-Alg scaffolds: The pore sizes of ^{0}PMO-Alg, $^{0.3}$PMO-Alg and $^{0.9}$PMO-Alg scaffolds were determined using ImageJ software, and for porosity measurements we used the ethanol replacement method. Therefore, dried scaffolds were immersed in absolute ethanol and kept overnight and weighed after removing the excess ethanol from the surface. The porosity was calculated from the following equation:

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

where M_1 and M_2 are the masses of scaffolds before and after being immersed in absolute ethanol, respectively, ρ is the density of absolute ethanol and V is the volume of the samples.

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 (|η*|) were measured from an amplitude sweep of ^{0}PMO-Alg, $^{0.3}$PMO-Alg and $^{0.9}$PMO-Alg 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, and the temperature was fixed at 25°C for all experiments.

General procedure for the co-staining of cells in the 3D-printed scaffolds: The morphology of cells on the samples was determined by Nikon ECLIPSE Ts2R fluorescence microscopy. After the incubation times, PFA 4% was added to each cell-embedded scaffold and kept for 10 min then washed 2 times with PBS. Hoechst 33342 dye was added to these samples for cell nucleus staining. In this case, the stock staining solution (16.2 mM) was made first, and it was diluted to 1:2000 by PBS before adding it to the cells; then cells were incubated for 10 min at room temperature, and they were washed again twice with PBS. Later on, these samples were kept in 0.1% Triton X-100 for 10 min at room temperature, cells were washed 2 times with PBS, and afterwards they were co-stained for f-actin by diluting 5 µl methanolic stock solution (6.6 µM) of Phalloidin Alexa Fluor 488 into 200 µl of PBS containing 3% bovine serum albumin (BSA); these samples were stored overnight in the dark at room temperature. After that, they were washed 2 times with PBS.

General procedure for double-staining cells with calcein AM and propidium iodide Calcein: AM/propidium iodide staining were used to investigate the cellular viability or cell death, respectively. For this purpose, 1 µM calcein AM and 1.5 µM propidium iodide were added at the same time into ^{0}PMO-Alg and $^{0.9}$PMO-Alg in the presence of fibroblast and Colo 818 cells, the samples were kept in incubator at 37°C and 5% CO2 for 30 min, then they were checked by a fluorescence microscope.

General procedure for the cell viability % in the 3D-printed step-gradient NC scaffolds (^{2}GradNS): The Fibroblast and Colo cells were carefully thawed and suspended in their specific medium (10% FBS + RPMI 1640). Then the cells were seeded homogeneously onto each part or only onto the ^{0}PMO-Alg part or only onto the $^{0.9}$PMO-Alg part of the ^{2}GradNS (approximately 10,000 cells for each scaffold). The ^{2}GradNS was covered with cell culture media (200 µl) and incubated for 1 hour, 1 day, 4 days and 7 days at 37°C and 5% CO_2. After the incubation periods, scaffolds were washed twice with phosphate-buffered saline (PBS) to remove non-adhered cells. Afterwards, the ^{2}GradNS was split into its layers (^{0}PMO-Alg and $^{0.9}$PMO-Alg), and the layers were transferred to new cell culture plates and treated with EDTA (0.04% w/v in PBS, without Ca^{2+}/Mg^{2+}) with gentle mixing. The cells were counted

immediately using a Neubauer chamber and the cell viability % was determined using trypan blue solution.

Characterization. The morphologies of the ^{0}PMO-Alg, $^{0.3}$PMO-Alg and $^{0.9}$PMO-Alg scaffolds were investigated using a Zeiss 1540 EsB dual beam focused ion beam/field emission SEM. Zeta potential measurements and dynamic light scattering (DLS) were measured by Malvern Zetasizer Nano Series. The morphologies of cells in hydrogels were determined using Nikon ECLIPSE Ts2R fluorescence microscopy. Inkredible 3D bioprinter (CELLINK) was used to print all scaffolds into computer-designed 3D structures. Anton Paar (Modular Compact Rheometer) was used for rheological analyses. The ANOVA test was used for statistical analyses. The number of particles on the surface areas of $^{0.3}$PMO-Alg and $^{0.9}$PMO-Alg scaffolds was determined using ImageJ software.

Table S1. Swelling ratio of ^{0}PMO-Alg, $^{0.3}$PMO-Alg and $^{0.9}$PMO-Alg after 1 day, 4 days and 7 days incubation at 37°C, [number of repeated experiments (N) = 3].

	PMO-Alg	**$^{0.3}$PMO-Alg**	**$^{0.9}$PMO-Alg**
1d	35.3 ± 1.3	59.1 ± 1.2	64.0 ± 1.0
4d	36.0 ± 1.3	59.6 ± 0.5	64.5 ± 1.5
7d	36.1 ± 0.	59.8 ± 0.3	64.6 ± 1.7

Table S2. Porosity (%) and pore size (µm) of ^{0}PMO-Alg, $^{0.3}$PMO-Alg and $^{0.9}$PMO-Alg.

	PMO-Alg	**$^{0.3}$PMO-Alg**	**$^{0.9}$PMO-Alg**
Porosity	46.7 ± 4.5	50.7 ± 5.3	56.7 ± 6.4
Pore size	258.9 ± 38.3	166.8 ± 28.2	123.3 ± 22.4

Table S3. Weight loss (%) of ^{0}PMO-Alg, $^{0.3}$PMO-Alg and $^{0.9}$PMO-Alg after 1 day, 4 days and 7 days incubation in the presence of and without cells at 37°C, [number of repeated experiments (N) = 3].

In presence of cells	**PMO-Alg**	**$^{0.3}$PMO-Alg**	**$^{0.9}$PMO-Alg**
1d	3.9 ± 0.1	2.3 ± 0.3	1.5 ± 0.4

4d	4.4 ± 0.1	2.6 ± 0.2	1.7 ± 0.4
7d	4.9 ± 0.2	2.9 ± 0.1	1.9 ± 0.4
W/O cells	**PMO-Alg**	**$^{0.3}$PMO-Alg**	**$^{0.9}$PMO-Alg**
1d	3.5 ± 0.4	1.7 ± 0.2	1.0 ± 0.5
4d	4.0 ± 0.3	2.3 ± 0.3	1.4 ± 0.4
7d	4.4 ± 0.4	2.7 ± 0.2	1.7 ± 0.1

Table S4. PMO distribution on 1 μm^2 area of Alg, [number of repeated experiments (N) = 3].

	Number of PMOs on 1 μm^2 area of Alg
$^{0.3}$PMO-Alg	6.03 ± 4.34
$^{0.9}$PMO-Alg	17.93 ± 3.70

Table S5. The percentage of Dex released from $^{0.3}$PMO-Alg and $^{0.9}$PMO-Alg at pH 7.4 and pH 6.0 at different time points.

	$^{0.3}$PMO-Alg pH 6.0	**$^{0.3}$PMO-Alg pH 7.4**	**$^{0.9}$PMO-Alg pH 6.0**	**$^{0.9}$PMO-Alg pH 7.4**
1 day	6.6 (%) ± 0.6	3.1 (%) ± 0.30	16.2 (%) ± 0.4	7.0 (%) ± 0.05
2 days	7.2 (%) ± 0.7	3.4 (%) ± 0.3	17.7 (%) ± 0.4	7.4 (%) ± 0.06
3 days	7.8 (%) ± 0.7	3.6 (%) ± 0.3	19.3 (%) ± 0.4	8.1 (%) ± 0.1
7 days	8.5 (%) ± 0.8	4.1 (%) ± 0.3	20.9 (%) ± 0.5	9.0 (%) ± 0.2

Table S6. Cell amounts (×10^3) when cells were seeded separately into the ^{0}PMO-Alg, $^{0.3}$PMO-Alg and $^{0.9}$PMO-Alg for incubation times of 1 hour, 1 day, 4 days and 7 days.

	^{0}PMO-Alg	**$^{0.3}$PMO-Alg**	**$^{0.9}$PMO-Alg**
1h	9.5 ± 0.5	9.7 ± 0.5	9.6 ± 0.4
1d	8.4 ± 0.6	12.2 ± 0.4	13.2 ± 0.3
4d	9.6 ± 0.3	14.9 ± 0.4	18.3 ± 0.4
7d	9.8 ± 0.4	15.4 ± 0.5	19.6 ± 0.3

Table S7. Cell amounts (×10^3) when cells were seeded into all parts, into just the ^{0}PMO-Alg part, and into just the $^{0.9}$PMO-Alg part of ^{3}GradNS for incubation times of 1 hour, 1 day, 4 days and 7 days.

A	Fibroblast cells in all parts		
	^{0}PMO-Alg	$^{0.3}$PMO-Alg	$^{0.9}$PMO-Alg
1h	9.4 ± 0.4	9.6 ± 0.4	9.7 ± 0.2
1d	7.5 ± 0.7	11.9 ± 0.5	13.6 ± 0.8
4d	8.0 ± 0.4	14.2 ± 0.4	18.5 ± 0.4
7d	7.8 ± 0.2	15.1 ± 0.3	19.9 ± 0.3
B	**Fibroblast cells in the ^{0}PMO-Alg part**		
	^{0}PMO-Alg	$^{0.3}$PMO-Alg	$^{0.9}$PMO-Alg
1h	9.5 ± 0.8	0	0
1d	9.0 ± 0.6	0.07 ± 0.01	0
4d	8.2 ± 0.6	0.7 ± 0.4	0.3 ± 0.3
7d	7.4 ± 0.5	1.4 ± 0.5	0.6 ± 0.2
C	**Fibroblast cells in the $^{0.9}$PMO-Alg part**		
	^{0}PMO-Alg	$^{0.3}$PMO-Alg	$^{0.9}$PMO-Alg
1h	0	0	9.5 ± 0.4
1d	0.9 ± 0.3	1.5 ± 0.2	14.0 ± 0.7
4d	1.7 ± 0.6	8.0 ± 0.6	17.2 ± 0.3
7d	2.4 ± 0.3	10.4 ± 0.3	19.4 ± 0.4

Table S8. Cell amounts ($\times 10^3$) when cells were seeded into all parts, into just the ^{0}PMO-Alg part and into just the $^{0.9}$PMO-Alg part of ^{2}GradNS for incubation times of 1 hour, 1 day, 4 days and 7 days.

A	Fibroblast cells in all parts		Colo 818 cells in all parts	
	^{0}PMO-Alg	$^{0.9}$PMO-Alg	^{0}PMO-Alg	$^{0.9}$PMO-Alg
1h	9.0 ± 0.3	9.4 ± 0.4	9.1 ± 0.3	9.4 ± 0.5
1d	8.2 ± 0.6	14.8 ± 0.6	9.0 ± 0.3	9.7 ± 0.6
4d	7.5 ± 0.5	19.4 ± 0.5	8.9 ± 0.3	10.6 ± 0.3
7d	7.2 ± 0.5	23.3 ± 0.6	8.4 ± 0.4	10.8 ± 0.3
B	**Fibroblast cells in the ^{0}PMO-Alg part**		**Colo 818 cells in the ^{0}PMO-Alg part**	
	^{0}PMO-Alg	$^{0.9}$PMO-Alg	^{0}PMO-Alg	$^{0.9}$PMO-Alg
1h	9.1 ± 0.5	0	9.1 ± 0.3	0
1d	8.8 ± 0.2	0.2 ± 0.1	9.1 ± 0.4	0.08 ± 0.05
4d	7.9 ± 0.4	1.9 ± 0.4	8.9 ± 0.2	1.1 ± 0.3
7d	7.6 ± 0.5	2.8 ± 0.6	8.5 ± 0.3	1.6 ± 0.3

C	Fibroblast cells in the $^{0.9}$PMO-Alg part		Colo 818 cells in the $^{0.9}$PMO-Alg part	
	^{0}PMO-Alg	$^{0.9}$PMO-Alg	^{0}PMO-Alg	$^{0.9}$PMO-Alg
1h	0	9.3 ± 0.5	0	9.3 ± 0.3
1d	0.4 ± 0.2	13.9 ± 0.5	0.4 ± 0.1	9.4 ± 0.3
4d	1.1 ± 0.4	18.1 ± 0.4	1.2 ± 0.5	10.3 ± 0.5
7d	1.9 ± 0.5	21.1 ± 0.5	2.0 ± 0.2	10.1 ± 0.3

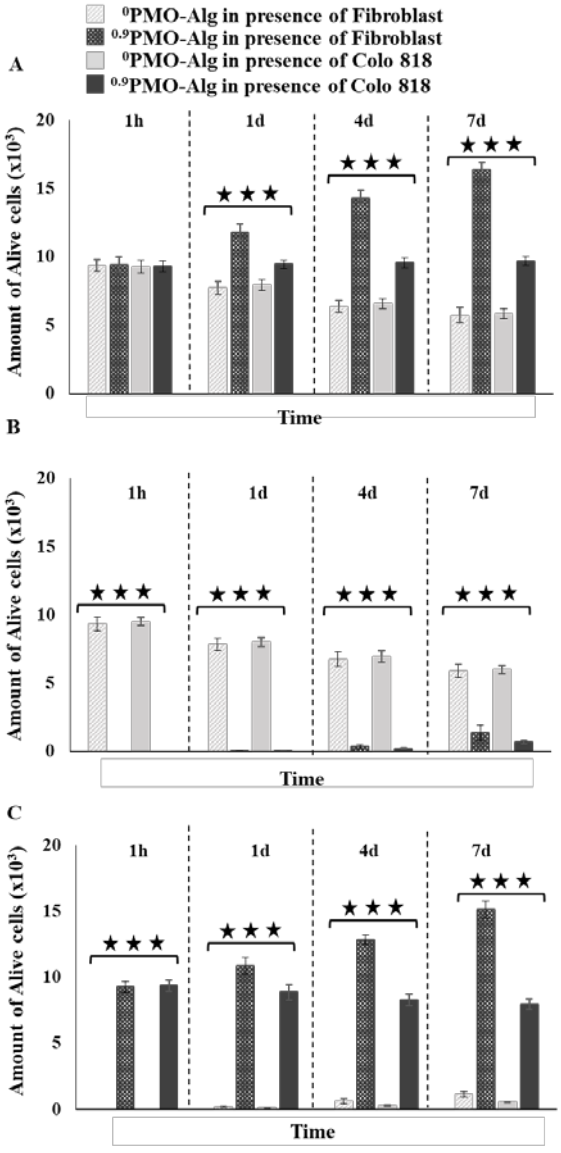

Figure S1. Cell amounts (×10^3) when cells were seeded into all parts (A), into just the ^{0}PMO-Alg part (B) and into just the $^{0.9}$PMO-Alg part (C) of ^{2}GradNS for incubation times of 1 hour, 1 day, 4 days and 7 days in presence of 1% FBS [number of experiments (N) = 3; data show significant differences; ANOVA: $p < 0.001$ (***)].

Table S9. Cell amounts ($\times 10^3$) when cells were seeded into all parts (A), into just the ^{0}PMO-Alg (B) part and into just the $^{0.9}$PMO-Alg (C) part of ^{2}GradNS for incubation times of 1 hour, 1 day, 4 days and 7 days in presence of 1% FBS.

A	**Fibroblast cells in all parts**		**Colo 818 cells in all parts**	
	^{0}PMO-Alg	**$^{0.9}$PMO-Alg**	**^{0}PMO-Alg**	**$^{0.9}$PMO-Alg**
1h	9.3 ± 0.4	9.4 ± 0.5	9.3 ± 0.5	9.3 ± 0.4
1d	7.7 ± 0.5	11.8 ± 0.6	7.9 ± 0.4	9.4 ± 0.3
4d	6.3 ± 0.4	14.3 ± 0.5	6.6 ± 0.4	9.5 ± 0.4
7d	5.7 ± 0.5	16.4 ± 0.5	5.8 ± 0.4	9.7 ± 0.3
B	**Fibroblast cells in the ^{0}PMO-Alg part**		**Colo 818 cells in the ^{0}PMO-Alg part**	
	^{0}PMO-Alg	**$^{0.9}$PMO-Alg**	**^{0}PMO-Alg**	**$^{0.9}$PMO-Alg**
1h	9.3 ± 0.5	0	9.5 ± 0.3	0
1d	7.8 ± 0.2	0.06 ± 0.02	8.0 ± 0.3	0.02 ± 0.01
4d	6.7 ± 0.5	0.4 ± 0.1	6.9 ± 0.4	0.2 ± 0.04
7d	5.9 ± 0.5	1.4 ± 0.5	6.0 ± 0.3	0.7 ± 0.1
C	**Fibroblast cells in the $^{0.9}$PMO-Alg part**		**Colo 818 cells in the $^{0.9}$PMO-Alg part**	
	^{0}PMO-Alg	**$^{0.9}$PMO-Alg**	**^{0}PMO-Alg**	**$^{0.9}$PMO-Alg**
1h	0	9.2 ± 0.4	0	9.4 ± 0.4
1d	0.2 ± 0.05	10.9 ± 0.3	0.1 ± 0.04	8.9 ± 0.5
4d	0.6 ± 0.4	12.8 ± 0.5	0.3 ± 0.07	8.3 ± 0.4
7d	1.1 ± 0.2	15.1 ± 0.3	0.5 ± 0.02	7.9 ± 0.4

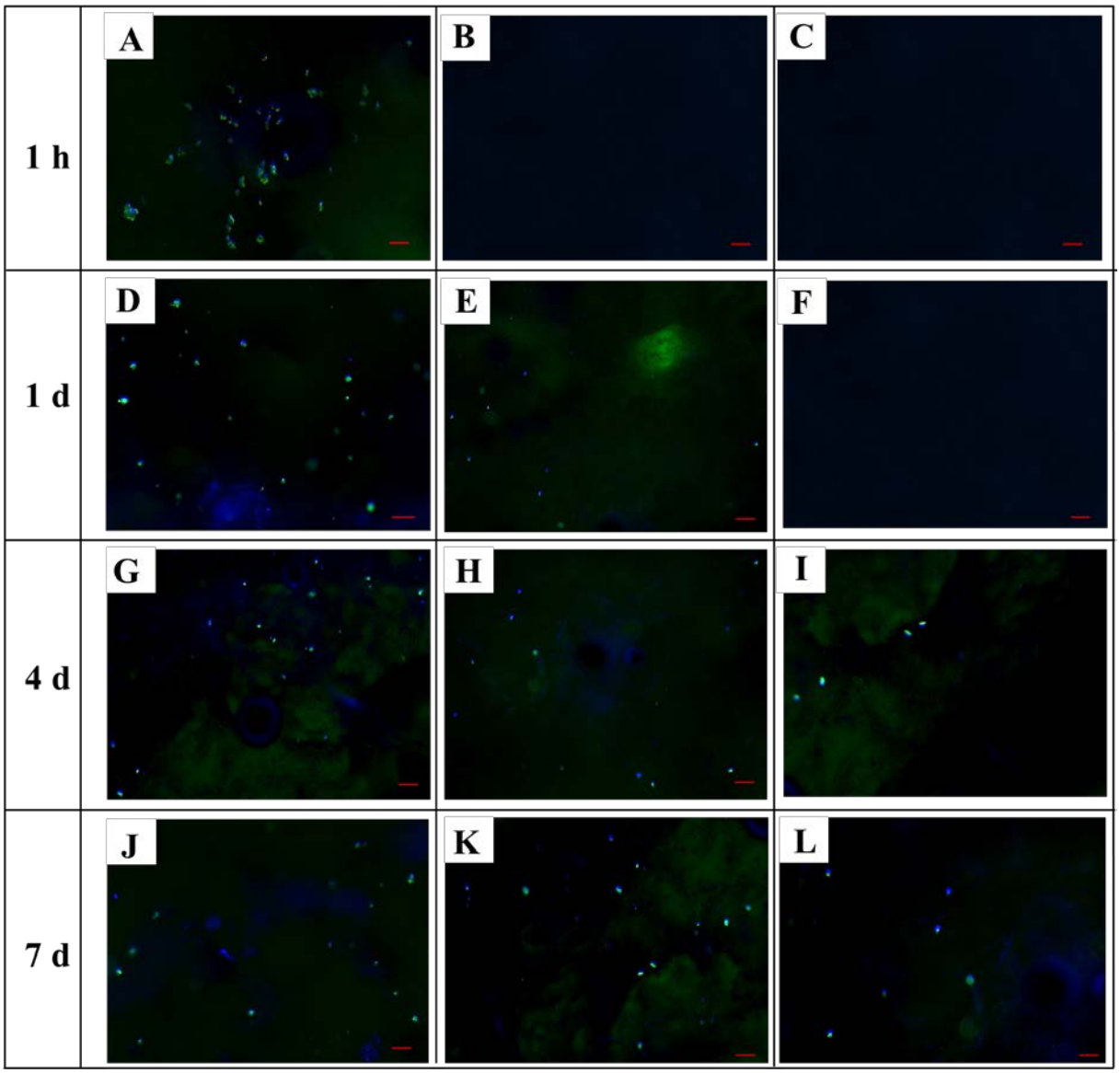

Figure S2. Fluorescence microscopy images (lower magnification) of fibroblast cells in the ^{0}PMO-Alg (A, D, G and J), $^{0.3}$PMO-Alg (B, E, H and K) and $^{0.9}$PMO-Alg (C, F, I and L) parts of ^{3}GradNS after 1 hour, 1 day, 4 days, and 7 days of incubation when cells were added just into the ^{0}PMO-Alg part with 4x magnification (the red lines shows the scale bar between 50-60 µm).

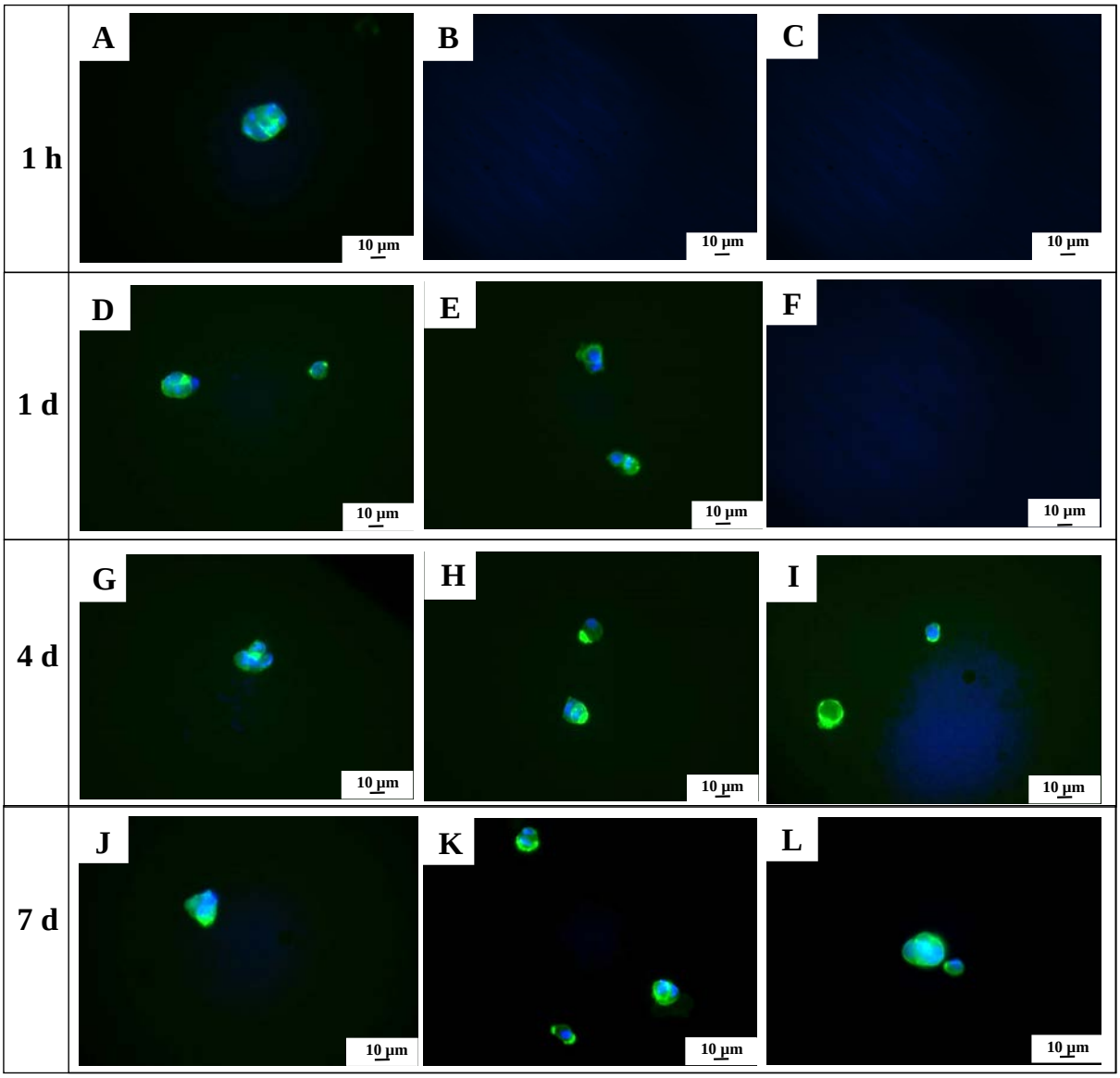

Figure S3. Fluorescence microscopy images (20x magnification) of fibroblast cells in the ^{0}PMO-Alg (A, D, G and J), $^{0.3}$PMO-Alg (B, E, H and K) and $^{0.9}$PMO-Alg (C, F, I and L) parts of ^{3}GradNS after 1 hour, 1 day, 4 days, and 7 days of incubation when cells were added just into the ^{0}PMO-Alg part.

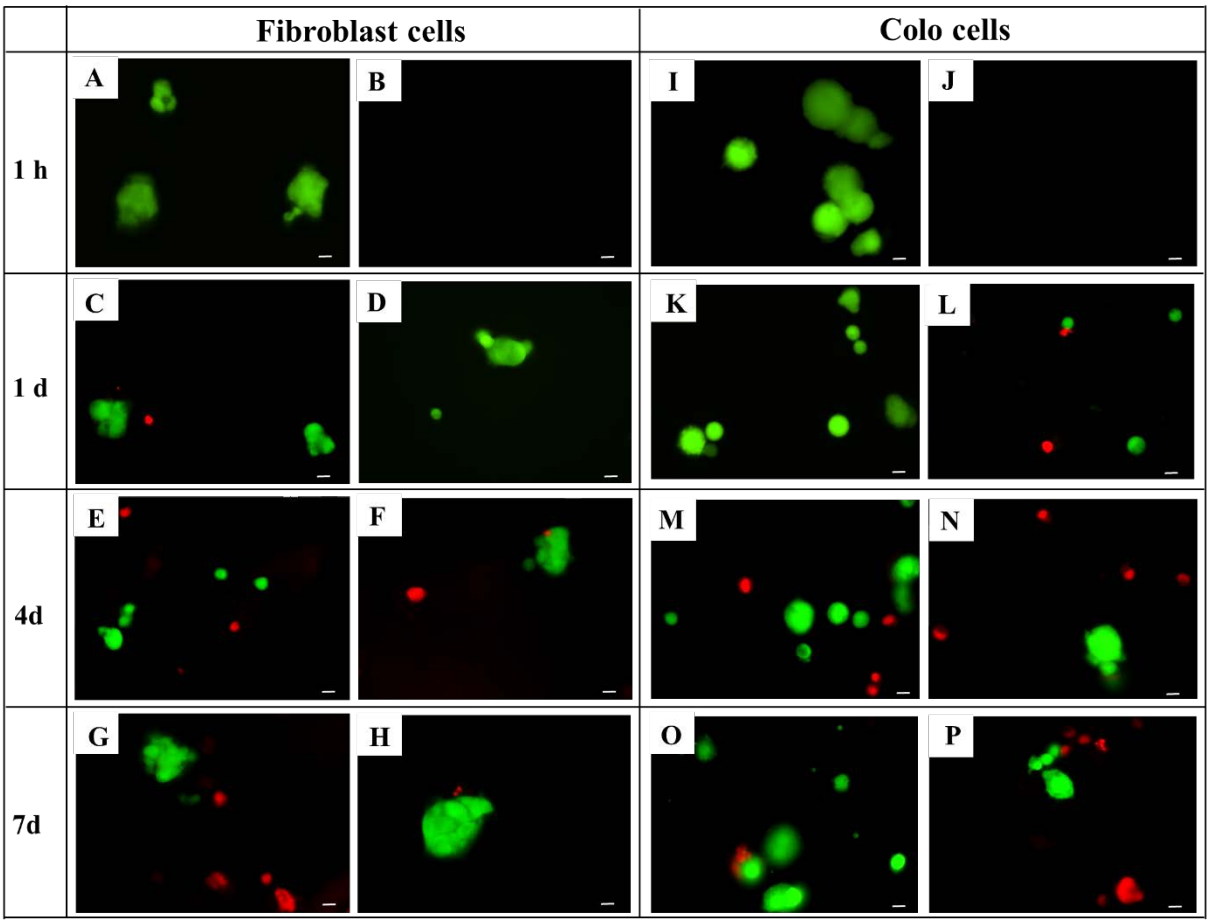

Figure S4. Fluorescence microscopy images of fibroblast cells in the ^{0}PMO-Alg (A, C, E and G) and $^{0.9}$PMO-Alg (B, D, F and H) parts of ^{2}GradNS after 1 hour, 1 day, 4 days, and 7 days of incubation when cells were added just into the ^{0}PMO-Alg part. Magnification is 40× and scale bar is 10 µm for all samples (red: dead cells by propidium iodide; green: alive cells by calcein AM).

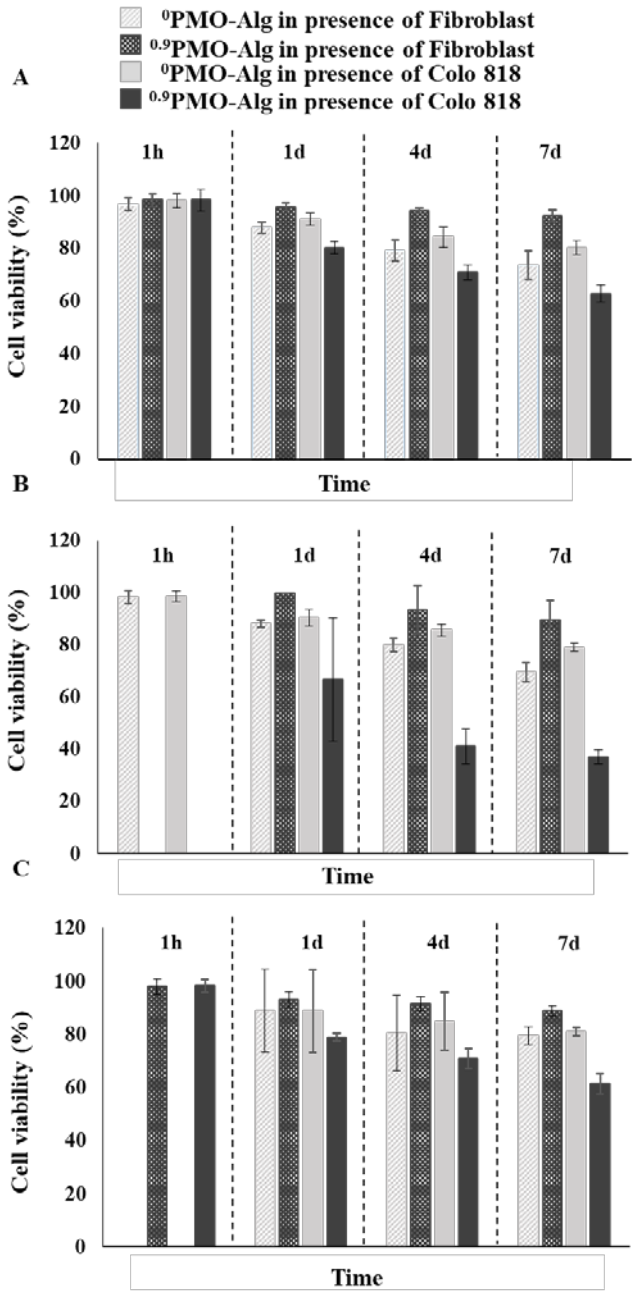

Figure S5. The cell viability (%) of cells that were seeded into all parts (A), into just the ^{0}PMO-Alg (B) part and into just the $^{0.9}$PMO-Alg (C) part of ^{2}GradNS for incubation times of 1 hour, 1 day, 4 days and 7 days. [number of experiments (N) = 3].