A multilayered cancer-on-a-chip model to analyze the effectiveness of new-generation photosensitizers

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Supporting information

1. Analysis of A2780, HOF cell cultures and A2780/HOF co-culture by flow cytometry

HOF cells and A2780 cells were seeded on a standard 12-well culture plate and cultured as the two independent monolayer cultures or A2780/HOF co-culture. The cell cultures were daily monitored using flow cytometry. Fig. S1 shows changes in populations of cultured cells during five-day culture.

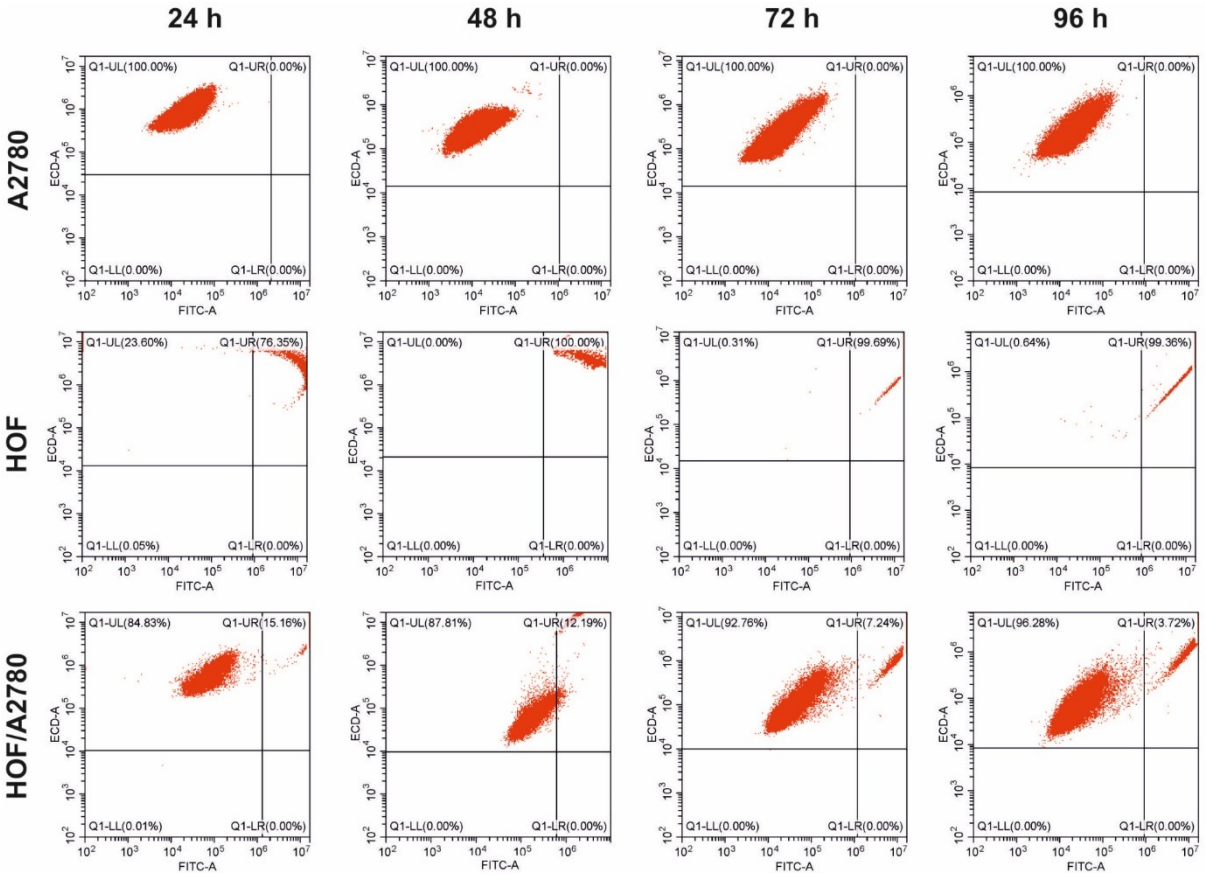

Fig. S1 The results of analysis of the population of A2780 and HOF cells cultured as monoculture and coculture obtained by flow cytometry. In the last row the ratio of the population of cancer cells

(A2780) and fibroblasts (HOF) in co-culture on the following days of culture was presented. 1st day ratio of A2780/HOF was 50% ÷ 50%.

It was found that the presence of fibroblasts stimulated growth of cancer cells. On 5th day of culture, the percent of cancer cells increased from 50 % to 96 % in co-culture. In addition, quantitative changes in the number of cells in the following populations: A2780 monoculture, HOF monoculture and A2780 / HOF co-culture were showed in Table 1.

Number of events in the population (in 100 ul of the sample):			
	A2780	**HOF**	**HOF/A2780**
24 h	7273	1958	10 806
48 h	16 382	1860	29 073
72 h	24 360	1923	52 547
96 h	38 590	2503	79 286

Table 1 Changes in the number of cells in populations: A2780, HOF and A2780/HOF in the following days of culture.

The results obtained using flow cytometry confirmed that after 96 hours of culture the number of non-malignant cells did not change significantly (Table 1). However, the number of cells cultured in the A2780/HOF culture on the last day of culture (96 h) increased about 8 times compared to the first day of culture (24 h). The observations confirmed previous conclusions that the presence of stromal cells affects the proliferation of cancer cells.

2. Efficiency of photodynamic therapy using nano-TPP – macroscale tests

Two types of ovarian cells: A2780 cancer cells and HOF non-malignant cells were seeded on standard 96-well plate at a density of 10^4 cells/well. The cells were cultured as monocultures (A2780 and HOF) and co-culture (A2780+HOF). To obtain the co-culture, non-malignant cells were mixed with cancer cells in a 1:1 ratio. Then the PDT procedure was performed. For this purpose, the solutions of nano-TPP (0 ÷ 10 μM) were added. After 24 h incubation of the cells with PS (37 °C, 5 %

CO_2), the cells were exposed to light (640 nm, 40 mW cm^{-2}). After 24 hours, cell viability was assessed by MTT test. Fig S2 shows the obtained results.

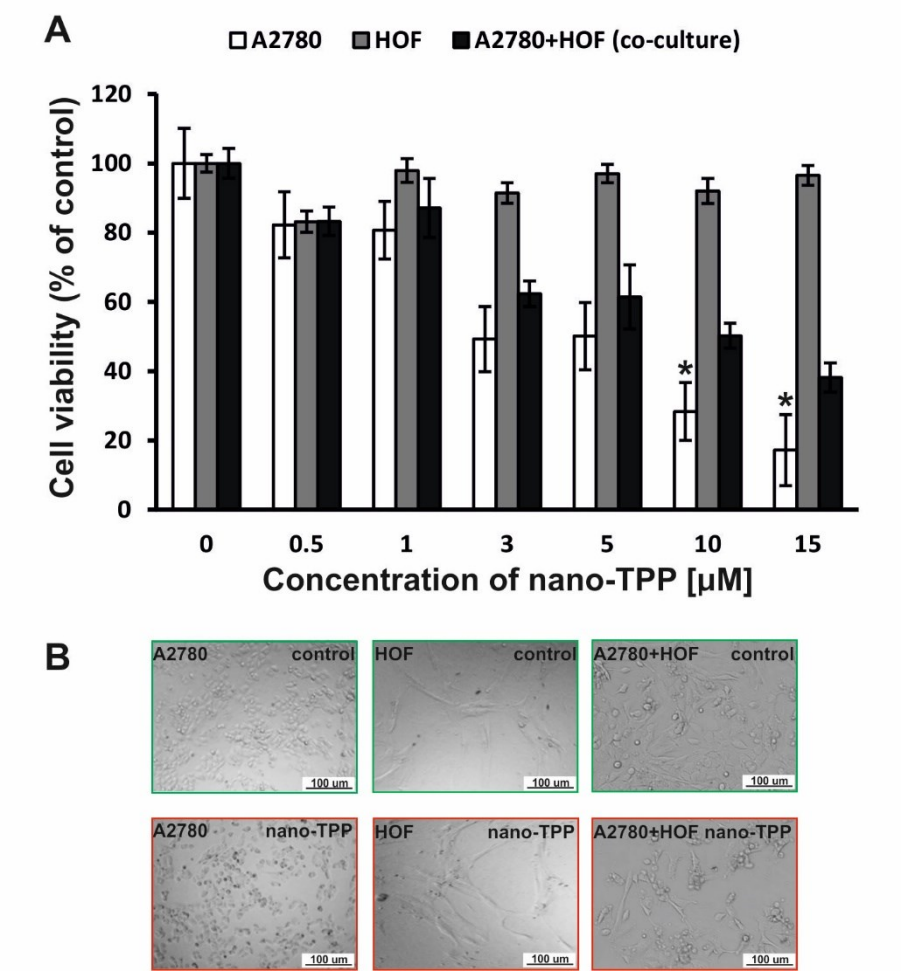

Fig. S2 A. The viability of A2780 cells, HOF cells and A2780+HOF cell co-culture after photodynamic therapy procedure with nano-TPP as a photosensitizer. Asterisks indicate statistically significant differences (ANOVA test, $\alpha = 0.05$), B. Microscopic images of A2780 cells, HOF cells and A2780+HOF co-culture after PDT procedure in the macroscale (for PS concentration = 5 µM).

The viability of non-malignant cells was high over the entire range of concentrations of tested PS. The viability of cancer cells decreased with the increasing of photosensitizer concentration. The results confirmed the selective photocytotoxicity of nanoencapsulated TPP (nano-TPP) on cancer cells. In the case of co-culture of A2780 and HOF cells, the total cell viability also decreased with increasing of PS concentration. For high drug concentrations: 5 µM, 10 µM and 15 µM, the cell viability in co-culture was: 61.37% ± 9.24%, 50.23% ± 3.62% and 38.17% ± 4.19%, respectively (Fig.

S2 A). In the case of microscale studies on a 3D cellular model, the total cell viability in co-culture already for concentration of 5 μM was lower than 40 % (Fig. 5 A).

The results obtained in the macroscale are different from the results obtained on 3D culture obtained by microfluidic system. In the standard 2D cell culture stromal cells and ovarian cancer cells grow side by side. However, in cell monolayer, the number of intercellular connections is lower than in 3D structure. Limitation of direct contact stroma-cancer may be the reason for weakened interactions between fibroblasts and cancer cells. Finally, in the macroscale conditions we can get a different response of cancer cells to the drug than in the microscale. Based on microscopic images, we conclude that after PDT procedure performed on cell co-culture, mainly cancer cells were dead, and the signal, which indicated the cell viability greater than in the microscale, came from live fibroblasts (Fig. S2 B). Probably in the macroscale, the non-malignant cells do not induce the activation of resistance mechanisms in cancer cells or stroma affect metabolism of PS in the other way, than under microfluidic conditions.

3. Comparison of cell viability in irradiated (LIGHT) and non-irradiated (DARK) controls

Two types of controls were used during the experiments in the microfluidic system. In the case of the photosensitizer cytotoxicity testing (Fig. 4A, Fig. 5A) control sample was the cells untreated with compound and untreated with a light. In the case of the photosensitizer photocytotoxicity studying (Fig. 4A, Fig. 5A) a control sample was the cells without photosensitizer (C_{PS} = 0 μM), but irradiated with LED. Differential cell staining in the irradiated and non-irradiated controls with Propidium Iodide and Calcein-AM was performed. The results are shown in Fig. S3.

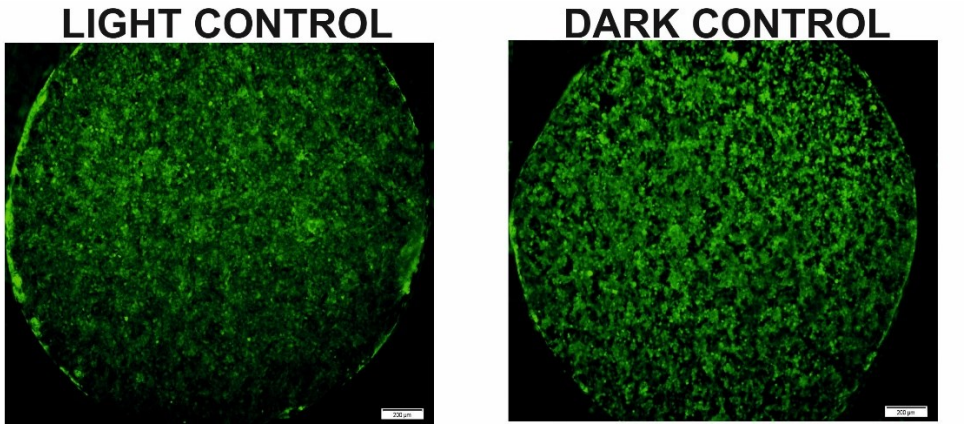

Fig. S3 Comparison of cell viability in irradiated (LIGHT) and non-irradiated (DARK) controls (green color - live cells, red color - dead cells).

The light used in PDT was not cytotoxic. No differences in cell viability were observed in the irradiated and non-irradiated controls. In both cases, the cell viability was about 100 %.