

Supplementary Materials: In Vitro Bioeffect of Polyelectrolyte Multilayer Microcapsules Post-Loaded with Water-Soluble Cationic Photosensitizer

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1. Temperature-induced morphological changes in the [DS/PAGr]₄ PMC system

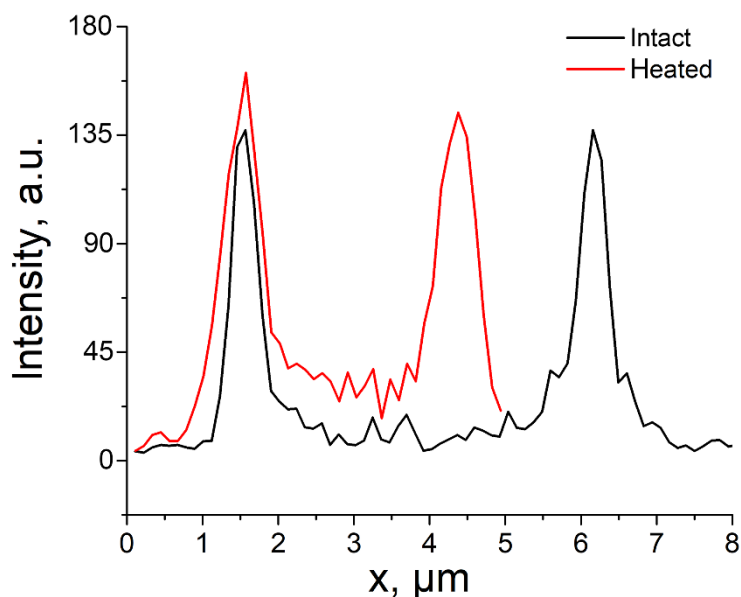


Figure S1. Fluorescence intensity cross-section profiles of the intact and heated capsules labeled with RhD6G ($\lambda_{\text{ex}}=525 \text{ nm}$, $\lambda_{\text{em}}=548 \text{ nm}$). The corresponding image was captured on the focal plane, allowing to visualize the maximum distance between two antipodal points on their surfaces. The capsule diameter is measured as a distance between the positions of maximums of two peaks.

2. Optical properties of Cholosens

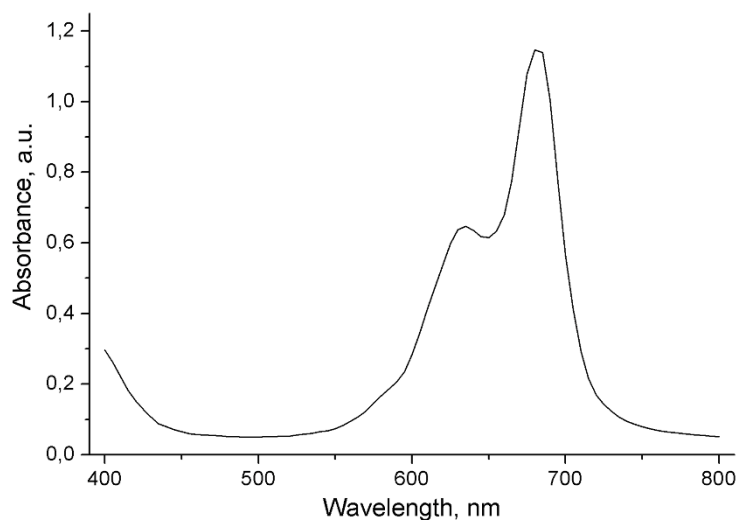


Figure S2. The absorbance spectrum of Cholosens. The spectrum was obtained in Di water (18.2 $\text{M}\Omega\text{cm}^{-1}$) at pH 6.6. Before measurements, the capsules were thoroughly washed via three sequential centrifugation steps, each followed by the exchanging of the supernatant.

3. Loading of Cholosens in PMC: loading data based on the analysis of enzymatically degraded capsules.

Samples of lyophilized Cholosens-loaded capsules loaded as displayed in Figure 3 were each mixed with 1 mL of 1 mg/mL α -chymotrypsin dissolved in 1 \times PBS (pH 7.4) in 2 mL centrifuge tubes and kept at 37 $^{\circ}\text{C}$ for 24 hours. Undissolved polymeric complexes were then extracted by centrifugation and resuspended again in the enzyme solution. The respective supernatants were then analyzed with fluorescence spectroscopy to determine the amount of Cholosens released from the capsules after the complete digestion of the PMC. The respective measured released data and calculated loading of Cholosens per a single capsule are shown in Figure S3.

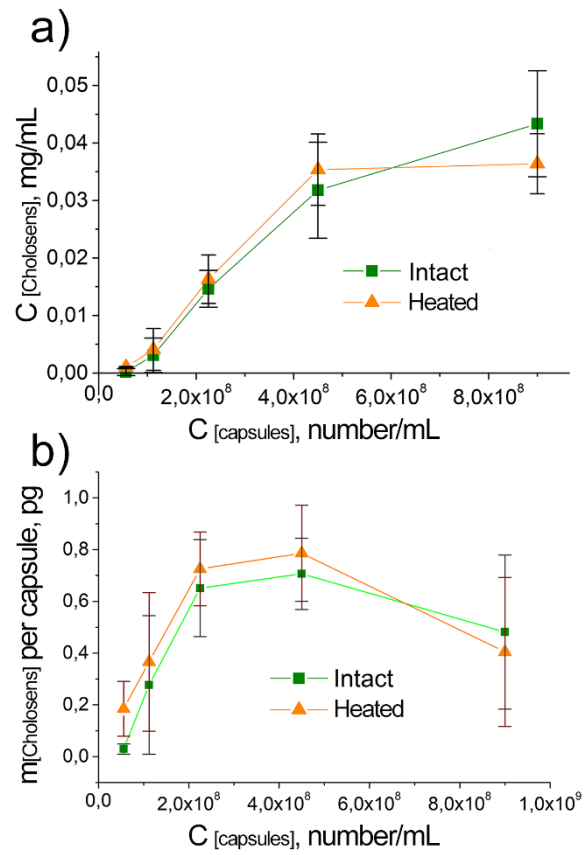


Figure S3. Loading of Cholosen in [DS/PArg]₄ microcapsules with and without heat treatment (80 °C, 60 min) depending on the capsule concentration: (a) spectroscopically measured concentration of Cholosen in the supernatant after the capsule enzymatic degradation; (b) calculated amount of Cholosen in pg released from a single capsule.

3. Student's test results

3.1. Student's test results for the cellular uptake of [DS/PArg]₄ microcapsules by HeLa cells (Figure 6 a)

The number of measurements was 3 for all uptake experiments; a p-value < 0.05 was considered statistically significant. Determination of the statistical significance of the differences between cellular uptake of the intact and heated PMC is presented in Table S1.

Table S1. Flow cytometry data on the capsules internalization by HeLa cells and calculated t parameter (n.s. means non-significant).

Time of incubation, h	Cells with Intact microcapsules, %	Cells with Heated microcapsules, %	t	degrees of freedom, df	t crit (* p<0.05)	result
1	32±3.5	42.6±4.4	3.2655	4	2.7764	*
2	41.5±5.2	52±3	3.0294			*
4	55±6	57.8±3.9	0.6777			n.s.
24	78.3±7.2	81.8±5.9	0.6512			n.s.

2.2. Student's test results for light and dark viability of HeLa and NHDF cells in the presence of free and encapsulated forms of Cholosens (Figure 7)

Table S2. Viability of HeLa and NHDF after treatment with 1 or 5 capsules per cell after irradiation (information for Fig.7b). Each point indicates the mean± (n=6).

<i>HeLa, 1 capsules/cell</i>						
With heated capsules, irradiated	In Cholosens solution, irradiated	t	df	t crit (* p<0.05)	t crit (** p<0.01)	result
39.76±0.77	33.94±0.72	13.5233	10	2.2281	3.1693	**
With intact capsules, irradiated		8.0051				**
39.26±1.46						
<i>HeLa, 5 capsules/cell</i>						
With heated capsules, irradiated	In Cholosens solution, irradiated	t	df	t crit (* p<0.05)	t crit (** p<0.01)	result
10.79±2.16	4.47±1.18	6.2897				**
With intact capsules, irradiated		1.0544				n.s.
5.02±0.49						
<i>NHDF, 1 capsules/cell</i>						
With heated capsules, irradiated	In Cholosens solution, irradiated	t	df	t crit (* p<0.05)	t crit (** p<0.01)	result
39.00±0.92	32.14±0.72	14.3835				**
With intact capsules, irradiated		10.3637				**
38.28±1.26						
<i>NHDF, 5 capsules/cell</i>						
With heated capsules, irradiated	In Cholosens solution, irradiated	t	df	t crit (* p<0.05)	t crit (** p<0.01)	result
4.62±0.06	3.02±0.32	12.0377				**
With intact capsules, irradiated		3.2268				**
4.07±0.73						

2.58±0.10	2.88±0.08	5.7382				
With heated capsules, non-irradiated	With intact capsules, non-irradiated	0.2968				n.s.
111.10±9.28	108.92±15.41					
<i>HeLa, 40 capsules/cell</i>						
With heated capsules, irradiated	With intact capsules, irradiated	t				n.s.
2.86±0.04	3.03±0.53	0.7835				
With heated capsules, non-irradiated	With intact capsules, non-irradiated	1.9649				n.s.
95.90±9.34	104.84±6.08					
<i>HeLa, 80 capsules/cell</i>						
With heated capsules, irradiated	With intact capsules, irradiated	t				n.s.
2.99±0.12	2.92±0.28	0.5629				
With heated capsules, non-irradiated	With intact capsules, non-irradiated	0.9140				n.s.
107.82±0.71	104.76±8.17					
<i>NHDF, 1 capsules/cell</i>						
With heated capsules, irradiated	With intact capsules, irradiated	t	df			n.s.
39,00±0,92	38,28±1,26	1.1304	10			
With heated capsules, non-irradiated	With intact capsules, non-irradiated	1.0333				n.s.
87,96±3,51	89,87±2,86					
<i>NHDF, 5 capsules/cell</i>						
With heated capsules, irradiated	With intact capsules, irradiated	t	df			n.s.
4,62±0,06	4,07±0,73	1.8393	10			
With heated capsules, non-irradiated	With intact capsules, non-irradiated	2.5310				*
65,16±13,51	79,57±3,46					
<i>NHDF, 20 capsules/cell</i>						
With heated capsules, irradiated	With intact capsules, irradiated	t	df			n.s.
2,52±0,40	2,54±0,31	0.0968	10			
With heated capsules, non-irradiated	With intact capsules, non-irradiated	3.2666				**
66,51±16,93	42,33±6,49					
<i>NHDF, 40 capsules/cell</i>						
With heated capsules, irradiated	With intact capsules, irradiated	t	df			n.s.
2,43±0,03	2,50±0,35	0.4881	10			
With heated capsules, non-irradiated	With intact capsules, non-irradiated	1.1925				n.s.
33,06±3,98	38,16±9,69					
<i>NHDF, 80 capsules/cell</i>						
With heated capsules, irradiated	With intact capsules, irradiated	t	df			**
2,69±0,16	2,45±0,05	3.5070	10			
With heated capsules, non-irradiated	With intact capsules, non-irradiated	0.6624				
39.30±3.58	38.13±2.43					

Table S6. Viability of non-irradiated HeLa cells treated with 1 or 5 capsules per cell (for Figure 7b). Each point indicates the mean± (n=6). Both intact and heated Cholosens loaded PMC displayed significantly lower non-irradiated toxicity to HeLa cells compared to Cholosens solution (p<0.01).

<i>HeLa, 1 capsules/cell</i>						
With heated capsules, non-irradiated	In Cholosens solution, non-irradiated	t	df	t crit (* p<0.05)	t crit (** p<0.01)	result
93,18±2,19	75,30±0,65	19.1719	10	2,2281	3,1693	**
With intact capsules, non-irradiated	In Cholosens solution, non-irradiated	26.6966				**
99,78±2,15	75,30±0,65					
<i>HeLa, 5 capsules/cell</i>						
With heated capsules, non-irradiated	In Cholosens solution, non-irradiated					**
89,18±2,69	61,32±0,70	24.5514				**
With intact capsules, non-irradiated	In Cholosens solution, non-irradiated	41.1840				
105.78±2.55	61.32±0.70					

2.3. Student's test results for light and dark viability of bacterial cells depending on the number of Cholosens-loaded capsules (intact or heated) added to the culture medium

Table S7. Viability of gram-positive *S. aureus* bacterial cells, %. Each point indicates the mean± (n=6).

<i>3 capsules/cell</i>						
With heated capsules, irradiated	With intact capsules, irradiated	t	df	t crit (* p<0.05)	t crit (** p<0.01)	result
37.5±3.115	25±3.129	6.9348	10	2.2281	3.1693	**
In Cholosens solution, irradiated	With intact capsules, irradiated					**
7±3,115	25±3,129	9.9862				**
In Cholosens solution, irradiated	With heated capsules, irradiated					**
7±3.115	37.5±3.115	16.9591				**
With heated capsules, non-irradiated	With intact capsules, non-irradiated					**
82±4.115	69.7±6.115	4.0877				**
In Cholosens solution, non-irradiated	With intact capsules, non-irradiated					**
29±3.115	69.7±6.115	14.5270				**
In Cholosens solution, non-irradiated	With heated capsules, non-irradiated		**			
29±3.115	82±4.115	25.1544				
<i>9 capsules/cell</i>						
With heated capsules, irradiated	With intact capsules, irradiated	t				
7.8±2.264	5.6±2.078	1.7536				n.s.
In Cholosens solution, irradiated	With intact capsules, irradiated					
3.05±1.864	5.6±2.078	2.2376				*
In Cholosens solution, irradiated	With heated capsules, irradiated					
3.05±1.864	7.8±2.264	3.9675				**
With heated capsules, non-irradiated	With intact capsules, non-irradiated					
63±3.864	51±3.864	5.3790				**
In Cholosens solution, non-irradiated	With intact capsules, non-irradiated					
11±2.164	51±3.864	17.9301				**
In Cholosens solution, non-irradiated	With heated capsules, non-irradiated					

11±2.164	63±3.864	28.7609				**
<i>18 capsules/cell</i>						
With heated capsules, irradiated	With intact capsules, irradiated	t				
5.75±1.6675	4.25±2.1825	1.3377				n.s.
In Cholosens solution, irradiated	With intact capsules, irradiated					
1.875±1.0175	4.25±2.1825	2.4159				*
In Cholosens solution, irradiated	With heated capsules, irradiated					
1.875±1.0175	5.75±1.6675	4.8590				**
With heated capsules, non-irradiated	With intact capsules, non-irradiated					
51±3.1675	44±3.1675	3.8277				**
In Cholosens solution, non-irradiated	With intact capsules, non-irradiated					
6±0.99	44±3.1675	28.0481				**
In Cholosens solution, non-irradiated	With heated capsules, non-irradiated					
6±0.99	51±3.1675	33.2148				**
<i>27 capsules/cell</i>						
With heated capsules, irradiated	With intact capsules, irradiated	t				
1.6±0.898	0±1.31	2.4676				*
In Cholosens solution, irradiated	With intact capsules, irradiated					
0±1.298	0±1.31	0				n.s.
In Cholosens solution, irradiated	With heated capsules, irradiated					
0±1.298	1.6±0.898	2.4831				*
With heated capsules, non-irradiated	With intact capsules, non-irradiated					
43±3.898	33±4.398	4.1681				**
In Cholosens solution, non-irradiated	With intact capsules, non-irradiated					
0±0.898	33±4.398	18.0080				**
In Cholosens solution, non-irradiated	With heated capsules, non-irradiated					
0±0.898	43±3.898	26.3313				**

Table S8. Viability of gram-negative E. coli, %. Each point indicates the mean± (n=6).

<i>5 capsules/cell</i>						
With heated capsules, irradiated	With intact capsules, irradiated	t	df	t crit (* p<0.05)	t crit (** p<0.01)	result
81.65066±5.08253	68.39813±5.08253	4.5163	10	2.2281	3.1693	**
In Cholosens solution, irradiated	With intact capsules, irradiated					**
39.22372±5.08253	68.39813±5.08253	9.9422				**
In Cholosens solution, irradiated	With heated capsules, irradiated					**
39.22372±5.08253	81.65066±5.08253	14.4585				n.s.
With heated capsules, non-irradiated	With intact capsules, non-irradiated					**
95.06878±3.08253	93.49001±4.08253	0.7560				**
In Cholosens solution, non-irradiated	With intact capsules, non-irradiated					**
77.36143±4.08253	93.49001±4.08253	6.8427				**
In Cholosens solution, non-irradiated	With heated capsules, non-irradiated					**
77.36143±4.08253	95.06878±3.08253	8.4788				
<i>10 capsules/cell</i>						
With heated capsules,	With intact capsules,	t				

irradiated	irradiated				
72.22943±4.08253	54.58033±6.08253	5.9014			**
In Cholosens solution, irradiated	With intact capsules, irradiated				
34.82715±4.08253	54.58033±6.08253	6.6050			**
In Cholosens solution, irradiated	With heated capsules, irradiated				
34.82715±4.08253	72.22943±4.08253	15.8683			**
With heated capsules, non-irradiated	With intact capsules, non-irradiated				
91.98547±4.08253	86.29847±4.08253	2.4128			*
In Cholosens solution, non-irradiated	With intact capsules, non-irradiated				
67.70496±4.61147	86.29847±4.08253	7.3949			**
In Cholosens solution, non-irradiated	With heated capsules, non-irradiated				
67.70496±4.61147	91.98547±4.08253	9.6567			**
<i>20 capsules/cell</i>					
With heated capsules, irradiated	With intact capsules, irradiated	t			result
47.62523±6.08253	32.59798±5.58253	4.4585			**
In Cholosens solution, irradiated	With intact capsules, irradiated				
25.66831±5.08253	32.59798±5.58253	2.2483			*
Cholosens solution irradiated	With heated capsules, irradiated				
25.66831±5.08253	47.62523±6.08253	6.7853			**
With heated capsules, non-irradiated	With intact capsules, non-irradiated				
84.26291±6.08253	59.43421±6.08253	7.0702			**
In Cholosens solution, non-irradiated	With intact capsules, non-irradiated				
51.49234±6.38126	59.43421±6.08253	2.2067			n.s.
In Cholosens solution, non-irradiated	With heated capsules, non-irradiated				
51.49234±6.38126	84.26291±6.08253	9.1054			**
<i>80 capsules/cell</i>					
With heated capsules, irradiated	With intact capsules, irradiated	t			
28.15988±5.425	3.3132±1.11601	10.4210			**
In Cholosens solution, irradiated	With intact capsules, irradiated				
0.59694±4.08253	1.11601±3.3132	0.2418			n.s.
Cholosens solution irradiated	With heated capsules, irradiated				
0.59694±4.08253	28.15988±5.425	9.9440			**
With heated capsules, non-irradiated	With intact capsules, non-irradiated				
87.28108±8.08253	58.13652±8.08253	6.2456			**
In Cholosens solution, non-irradiated	With intact capsules, non-irradiated				

51.1809±7.311	58.13652±8.08253	1.5633				n.s.
In Cholosen solution, non-irradiated	With heated capsules, non- irradiated					
51.1809±7.311	87.28108±8.0825	8.1137				**

Table S9. The difference in viability of gram-positive *S. aureus* and gram-negative *E. coli*. (%) at different doses of capsules per cell

<i>S. aureus, With heated capsules, irradiated</i>						
3 capsules/cell	9 capsules/cell	t	df	t crit (* p<0.05)	t crit (** p<0.01)	result
37.5±3.115	7.8±2.264	18.8920	10	2,2281	3,1693	**
<i>S. aureus, With heated capsules, non-irradiated</i>						
3 capsules/cell	9 capsules/cell	t	df	t crit (* p<0.05)	t crit (** p<0.01)	result
82±4.115	63±3.864	8.2448				**
<i>S. aureus, With intact capsules, irradiated</i>						
3 capsules/cell	9 capsules/cell	t	df	t crit (* p<0.05)	t crit (** p<0.01)	result
25±3.129	5.6±2.078	12.6513				**
<i>S. aureus, With intact capsules, non-irradiated</i>						
3 capsules/cell	9 capsules/cell	t	df	t crit (* p<0.05)	t crit (** p<0.01)	result
69.7±6.115	51±3.864	6.3324				**
<i>S. aureus, in Cholosen solution, irradiated</i>						
3 capsules/cell	9 capsules/cell	t	df	t crit (* p<0.05)	t crit (** p<0.01)	result
7±3.115	3.05±1.864	2.6653				*
<i>S. aureus, in Cholosen solution, non-irradiated</i>						
3 capsules/cell	9 capsules/cell	t	df	t crit (* p<0.05)	t crit (** p<0.01)	result
29±3.115	11±2.164	11.6246				**
<i>E. coli., with heated capsules, irradiated</i>						
10 capsules/cell	20 capsules/cell	t	df	t crit (* p<0.05)	t crit (** p<0.01)	result
72.22943±4.08253	47.62523±6.08253	8.2270				**
<i>E. coli., with heated capsules, non-irradiated</i>						
10 capsules/cell	20 capsules/cell	t	df	t crit (* p<0.05)	t crit (** p<0.01)	result
91.98547±4.08253	84.26291±6.08253	2.5822				*
<i>E. coli., with intact capsules, irradiated</i>						
10 capsules/cell	20 capsules/cell	t	df	t crit (* p<0.05)	t crit (** p<0.01)	result
54.58033±6.08253	32.59798±5.58253	6.5220				**
<i>E. coli., with intact capsules, non-irradiated</i>						
10 capsules/cell	20 capsules/cell	t	df	t crit (* p<0.05)	t crit (** p<0.01)	result
86.29847±4.08253	59.43421±6.08253	8.9827				**
<i>E. coli., in Cholosen solution, irradiated</i>						
10 capsules/cell	20 capsules/cell	t	df	t crit (* p<0.05)	t crit (** p<0.01)	result
34.82715±4.08253	25.66831±5.08253	3.4413				**
<i>E. coli., in Cholosen solution, non-irradiated</i>						
10 capsules/cell	20 capsules/cell	t	df	t crit (* p<0.05)	t crit (** p<0.01)	result
67.70496±4.61147	51.49234±6.38126	5.0441				**

Table S10. IC50 for HeLa and NHDF cells

<i>Irradiated</i>						
Cell type	Intact capsules		Heated capsules		Free Cholosens	
	cap per cell	m[Cholosens], pg	cap per cell	m[Cholosens], pg	cap per cell equivalent	m[Cholosens], pg
HeLa	3.25	2.9	3.73	3.4	2.89	2.6
NHDF	3.1	2.8	3.2	2.88	2.64	2.4
<i>Non-irradiated</i>						
Cell type	Intact capsules		Heated capsules		Free Cholosens	
	cap per cell	m[Cholosens], pg	cap per cell	m[Cholosens], pg	cap per cell equivalent	m[Cholosens], pg
HeLa	Not established*	Not established*	Not established*	Not established*	9.1	8.2
NHDF	16,91	15,2	29.87	26.9	13.6	12.2

* The cell viability never drops below 50%

Table S11. IC50 for *S. aureus* and *E. Coli*

<i>Irradiated</i>						
Cell type	Intact capsules		Heated capsules		Free Cholosens	
	cap per cell	m[Cholosens], pg	cap per cell	m[Cholosens], pg	cap per cell equivalent	m[Cholosens], pg
<i>S. aureus</i>	0.85	0.77	0.94	0.846	Not established**	Not established**
<i>E. Coli</i>	12.2	10.8	17.1	26.9	Not established**	Not established**
<i>Non-irradiated</i>						
Cell type	Intact capsules		Heated capsules		Free Cholosens	
	cap per cell	m[Cholosens], pg	cap per cell	m[Cholosens], pg	cap per cell equivalent	m[Cholosens], pg
<i>S. aureus</i>	10.3	0.9	19.13	0.9	Not established**	Not established**
<i>E. Coli</i>	493***	443.7***	636***	572.4***	316***	284.4***

** The cell viability drops below 50%

*** Not practicable