

Mitochondria-Targeted Delivery Strategy of Dual-Loaded Liposomes for Alzheimer's Disease Therapy

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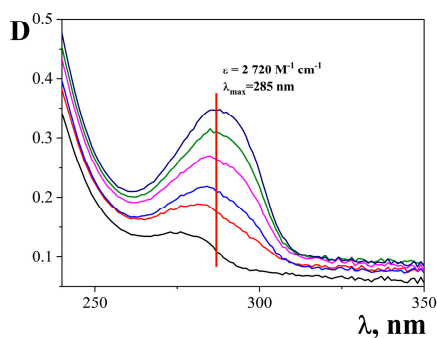
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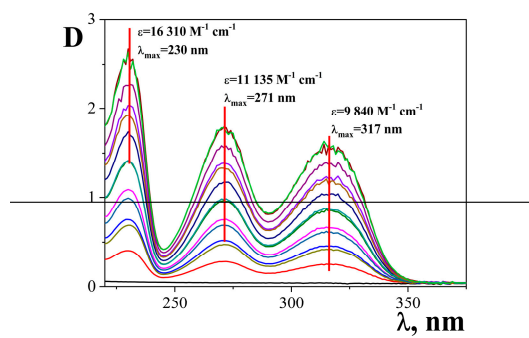
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Table S1. Physicochemical parameters of liposomes modified with TPPB-14. D_h – hydrodynamic diameter, PDI – polydispersity index, ζ – zeta potential, 4°C.

| Storage time | PC/Chol | | | PC/Chol/TPPB-14 | | | PC/Chol/TOC | | | PC/Chol/TOC/TPPB-14 | | |
|--------------|------------|---------------|--------------|-----------------|---------------|--------------|-------------|---------------|--------------|---------------------|---------------|--------------|
| | D_h , nm | PDI | ζ , mV | D_h , nm | PDI | ζ , mV | D_h , nm | PDI | ζ , mV | D_h , nm | PDI | ζ , mV |
| 15 mM | | | | | | | | | | | | |
| 1 day | 107 ± 2 | 0.071 ± 0.027 | -9.2 ± 1.2 | 105 ± 2 | 0.109 ± 0.010 | 8.2 ± 1.3 | 105 ± 2 | 0.067 ± 0.012 | -1.8 ± 0.8 | 110 ± 3 | 0.133 ± 0.011 | 6.8 ± 0.4 |
| 1 week | 116 ± 3 | 0.119 ± 0.001 | -12.3 ± 2.3 | 166 ± 2 | 0.234 ± 0.008 | 18.5 ± 1.9 | 130 ± 8 | 0.226 ± 0.015 | -7.1 ± 0.4 | 129 ± 3 | 0.209 ± 0.014 | 19.0 ± 2.6 |
| 2 months | 112 ± 2 | 0.097 ± 0.012 | -10.4 ± 0.7 | 113 ± 3 | 0.123 ± 0.014 | 22.5 ± 1.5 | 111 ± 2 | 0.130 ± 0.012 | -23.1 ± 1.2 | 115 ± 3 | 0.140 ± 0.011 | 22.1 ± 3.6 |
| 4 months | 114 ± 1 | 0.188 ± 0.009 | -10.3 ± 0.5 | 116 ± 1 | 0.124 ± 0.006 | 20.9 ± 1.3 | 113 ± 1 | 0.124 ± 0.024 | -13.2 ± 0.3 | 119 ± 1 | 0.122 ± 0.010 | 20.6 ± 0.5 |
| 5 months | 116 ± 4 | 0.116 ± 0.005 | -10.6 ± 1.7 | 115 ± 3 | 0.114 ± 0.014 | 24.3 ± 1.9 | 114 ± 3 | 0.115 ± 0.004 | -17.0 ± 1.4 | 121 ± 2 | 0.101 ± 0.010 | 22.7 ± 3.4 |
| 20 mM | | | | | | | | | | | | |
| 1 day | 108 ± 2 | 0.078 ± 0.006 | -7.6 ± 0.5 | 110 ± 2 | 0.193 ± 0.010 | 14.3 ± 2.2 | 104 ± 3 | 0.071 ± 0.01 | -2.9 ± 1.4 | 113 ± 2 | 0.231 ± 0.015 | 11.0 ± 1.0 |
| 1 week | 112 ± 1 | 0.079 ± 0.028 | -2.3 ± 0.5 | 118 ± 2 | 0.168 ± 0.00 | 24.8 ± 2.2 | 114 ± 1 | 0.154 ± 0.016 | -1.9 ± 0.5 | 114 ± 1 | 0.119 ± 0.018 | 32.3 ± 0.1 |
| 2 months | 127 ± 2 | 0.198 ± 0.017 | -3.9 ± 0.2 | 129 ± 5 | 0.202 ± 0.024 | 40.5 ± 4.3 | 108 ± 2 | 0.074 ± 0.015 | -8.4 ± 1.2 | 119 ± 4 | 0.155 ± 0.025 | 34.8 ± 1.5 |
| 4 months | 112 ± 1 | 0.092 ± 0.017 | -6.4 ± 1.9 | 114 ± 1 | 0.150 ± 0.003 | 35.9 ± 1.4 | 113 ± 1 | 0.088 ± 0.018 | -11.2 ± 3.3 | 130 ± 2 | 0.186 ± 0.005 | 27.0 ± 1.0 |
| 5 months | 116 ± 2 | 0.085 ± 0.008 | -1.6 ± 0.5 | 115 ± 1 | 0.114 ± 0.013 | 36.6 ± 0.5 | 116 ± 2 | 0.101 ± 0.005 | -7.9 ± 1.4 | 122 ± 1 | 0.105 ± 0.008 | 30.5 ± 0.3 |
| 30 mM | | | | | | | | | | | | |
| 1 day | 115 ± 2 | 0.067 ± 0.015 | -4.9 ± 0.5 | 115 ± 2 | 0.120 ± 0.01 | 23.9 ± 3.4 | 103 ± 2 | 0.077 ± 0.005 | -3.6 ± 0.7 | 110 ± 1 | 0.148 ± 0.017 | 11.3 ± 2.8 |
| 1 week | 115 ± 1 | 0.058 ± 0.039 | -5.5 ± 1.7 | 116 ± 1 | 0.103 ± 0.011 | 25.5 ± 1.8 | 105 ± 2 | 0.071 ± 0.017 | -5.8 ± 0.4 | 112 ± 2 | 0.141 ± 0.060 | 23.5 ± 2.1 |
| 2 months | 124 ± 1 | 0.127 ± 0.020 | -6.9 ± 1.2 | 120 ± 4 | 0.140 ± 0.023 | 41.1 ± 3.3 | 111 ± 4 | 0.138 ± 0.024 | -7.2 ± 1.2 | 112 ± 2 | 0.125 ± 0.018 | 31.9 ± 2.2 |
| 4 months | 121 ± 1 | 0.104 ± 0.023 | -14.4 ± 8.3 | 123 ± 2 | 0.121 ± 0.019 | 35.3 ± 0.9 | 126 ± 3 | 0.154 ± 0.023 | -7.2 ± 0.5 | 117 ± 1 | 0.121 ± 0.013 | 26.3 ± 2.9 |
| 5 months | 121 ± 1 | 0.083 ± 0.012 | -5.0 ± 0.9 | 126 ± 1 | 0.142 ± 0.029 | 35.1 ± 1.4 | 158 ± 1 | 0.147 ± 0.016 | -4.0 ± 0.2 | 120 ± 1 | 0.103 ± 0.021 | 27.3 ± 0.9 |



(a)



(b)

Figure S1. Absorption spectra and extinction coefficient of (a) α -tocopherol (in ethanol) and (b) donepezil hydrochloride (in Milli-Q water); cuvette thickness = 1 cm.

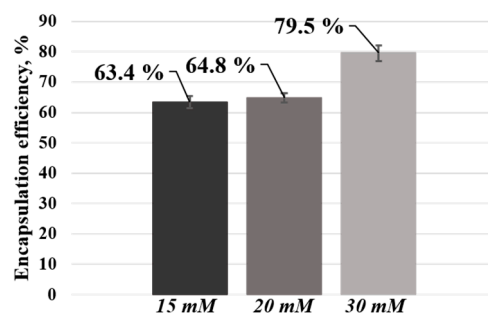
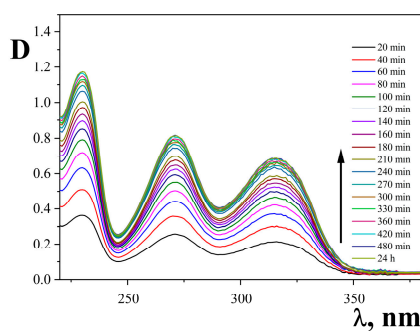
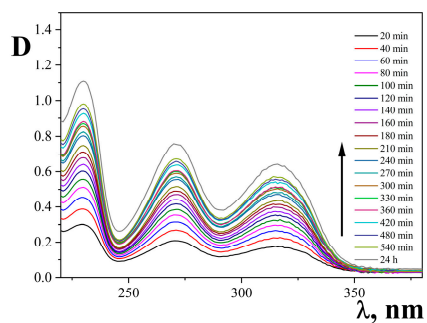


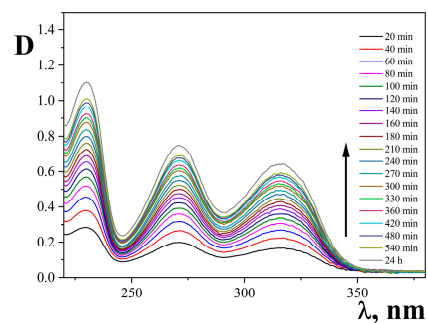
Figure S2. Encapsulation efficiency of PC/Chol/TPPB-14 liposomes toward donepezil hydrochloride.



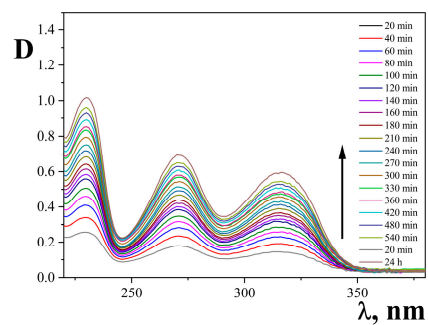
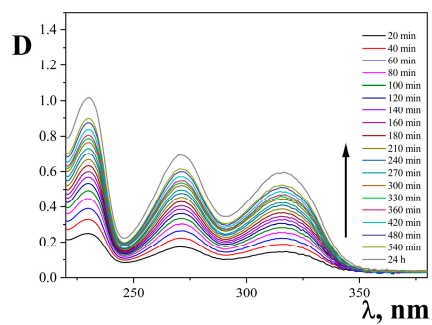
(a)



(b)



(c)



(d)

(e)

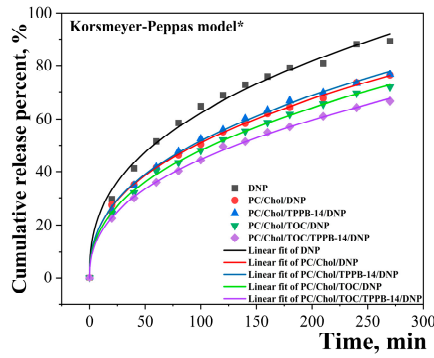
Figure S3. The absorption spectra of DNP at different time intervals of release for: (a) free DNP; (b) PC/Chol/DNP; (c) PC/Chol/TPPB-14/DNP; (d) PC/Chol/TOC/DNP; (e) PC/Chol/TOC/TPPB-14/DNP. Total lipid concentration is 20 mM. Phosphate buffer (0.025 M), pH = 7.4, 37°C; cuvette thickness = 1 cm; the arrow indicates the direction of dialysis duration increasing.

Table S2. The release kinetic model fitting parameters of DNP*.

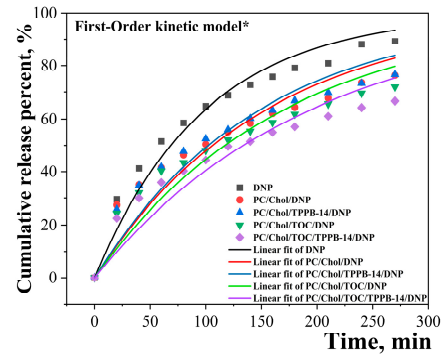
| System | Model | | | | | | | | |
|-----------------------|------------------|----------------------------------|----------------|------------------|----------------|---------------------------------|----------------|------------------|----------------|
| | Korsmeyer-Peppas | | | First-Order | | Higuchi | | Zero-Order | |
| | n | k_{KP} , %/min ⁿ | R ² | k_1 , 1/min | R ² | k_H , %/min ^{1/2} | R ² | k_0 , %/min | R ² |
| DNP | 0.392 | 10.234 | 0.9940 | 0.010 | 0.9533 | 5.929 | 0.9691 | 0.428 | 0.4570 |
| PC/Chol** | 0.403 | 7.977 | 0.9993 | 0.007 | 0.8775 | 4.881 | 0.9801 | 0.354 | 0.5065 |
| PC/Chol/TPPB-14** | 0.408 | 7.915 | 0.9999 | 0.007 | 0.8973 | 4.965 | 0.9815 | 0.360 | 0.5127 |
| PC/Chol/TOC** | 0.415 | 7.123 | 0.9992 | 0.006 | 0.8778 | 4.630 | 0.9848 | 0.336 | 0.5358 |
| PC/Chol/TOC/TPPB-14** | 0.417 | 6.539 | 0.9991 | – | – | 4.299 | 0.9855 | 0.312 | 0.5405 |

* Data obtained for the initial part of the DNP release curves, i.e., from 0 min to 270 min.

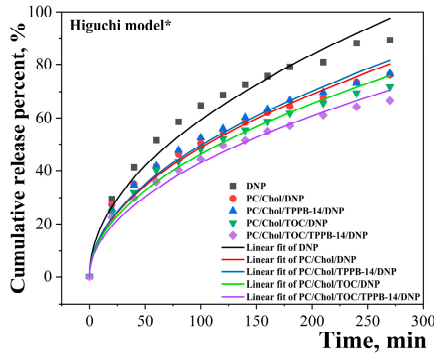
** Total lipid concentration is 20 mM.



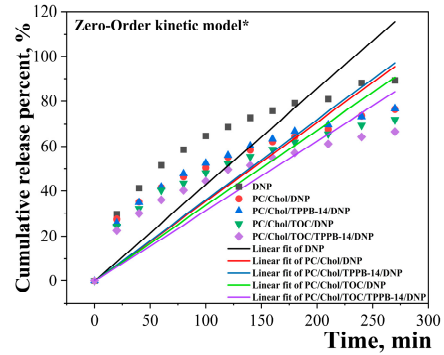
(a)



(b)



(c)



(d)

Figure S4. The release kinetic model fitting curves of DNP: (a) Korsmeyer–Peppas model; (b) First-Order kinetic model; (c) Higuchi model; (d) Zero-Order kinetic model. Total lipid concentration is 20 mM. Phosphate buffer (0.025 M), pH = 7.4, 37°C. * Data obtained for the initial part of the DNP release curves, i.e., from 0 min to 270 min.

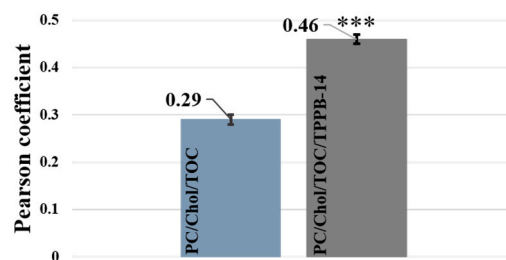


Figure S5. Pearson's Correlation Coefficient for modified and unmodified liposomes. Data are presented as means \pm SD. *** — difference with unmodified liposomes is statistically significant at $p \leq 0.001$. Statistical analysis was performed using the One-Way ANOVA test.

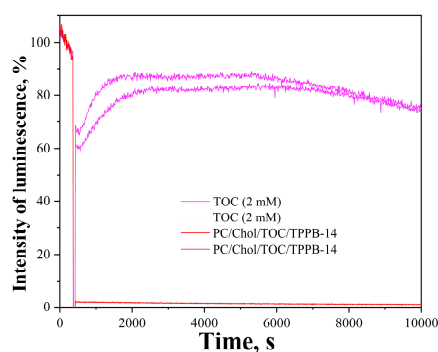


Figure S6. Dependences of chemiluminescence intensity on time. The experiment was carried out twice for each sample.

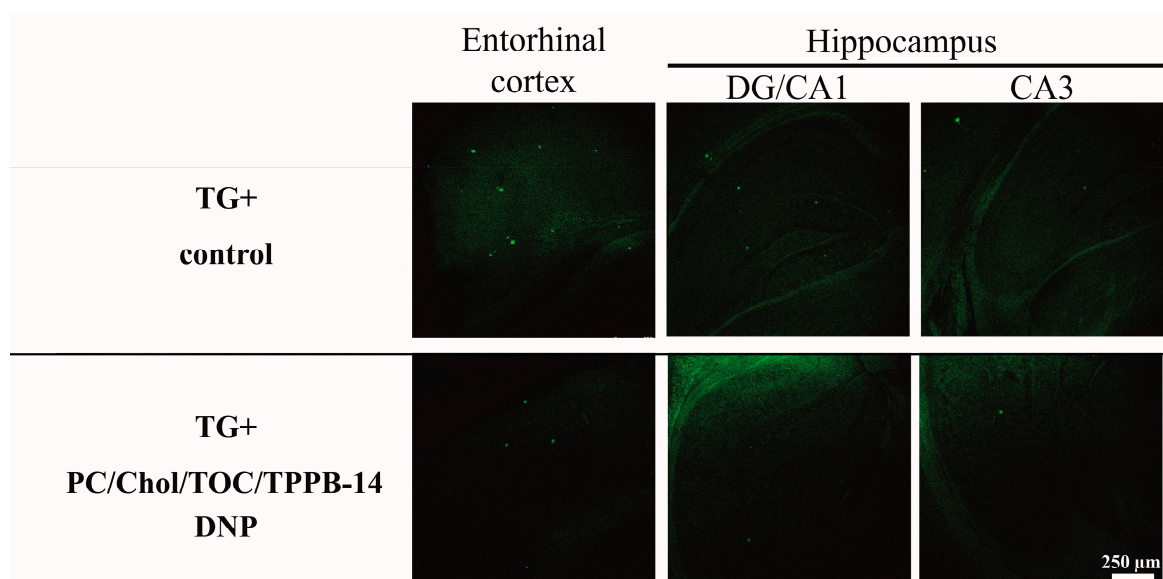


Figure S7. Visualization of A β plaques in the entorhinal cortex and areas of hippocampus of control group of transgenic (TG+) mice and group of transgenic (TG+) mice treated with PC/Chol/TOC/TPPB-14/DNP liposomes. Scale bar 250 μ m.