Electronic supplementary information

Mucin-targeting-aptamer functionalized liposomes for delivery of cyclosporin A for dry eye diseases

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Table S1. Primer sequences for real-time PCR to quantify mRNA expression of various genes.

Gene	Primer	Primer sequence (5'->3')
IL-1β	Forward	CCTGTCCTGCGTGTTGAAAGA
	Reverse	GGGAACTGGGCAGACTCAAA
IL-6	Forward	CACAGACAGCCACTCACCTC
	Reverse	TTTTCTGCCAGTGCCTCTTT
IL-8	Forward	TTTCAGAGACAGCAGAGCACACAA
	Reverse	CACACAGAGCTGCAGAAATCAGG
GAPDH	Forward	GAAGGTGAAGGTCGGAGTC
	Reverse	GAAGATGGTGATGGGATTTC

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Table S2. Quantification of CsA in combined liposomes.

	10 wt.%	20 wt.%	40 wt.%	50 wt.%
Total CsA				
Absorbance at 205 nm [CsA loaded liposome – liposome]	**0.2**	**0.3**	**0.2**	**0.3**
Total CsA (µg/ml) in liposome (5mg/ml)	**502.7**	**895.4**	**605.0**	**772.2**
Unloaded CsA				
Absorbance at 205 nm [CsA loaded liposome – liposome]	**0.01**	**0.03**	**0.06**	**0.08**
Unloaded CsA (µg/ml) in liposome (5mg/ml)	**10.1**	**17.1**	**23.8**	**29.9**
Drug loading content (%)				
(Weight of CsA in Liposome / Weight of liposome) x 100	**9.9**	**17.6**	**11.6**	**14.8**
Encapsulation efficiency (%)				
((Weight of total CsA – weight of unloaded CsA)/ Weight of initial CsA) x 100	**97.5**	**87.8**	**29.1**	**26.7**

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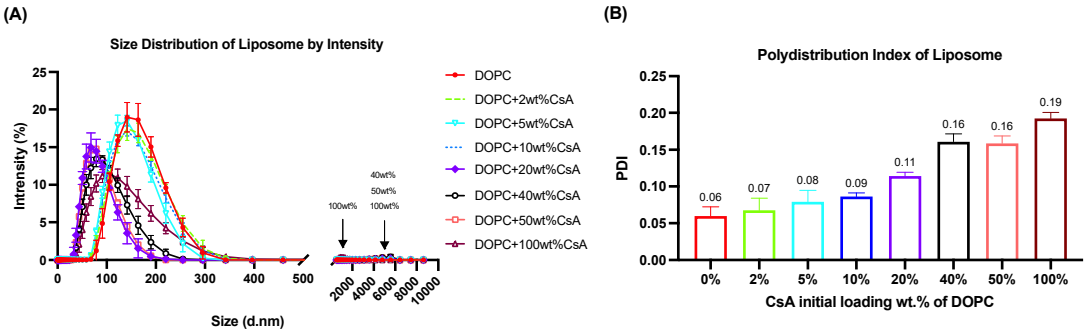

Figure S1. Size analysis of liposome by DLS. (A) The size distribution of liposomes and (B) PDI with different CsA loading by intensity. n=3. The measurements were performed in PBS at 25 ºC.

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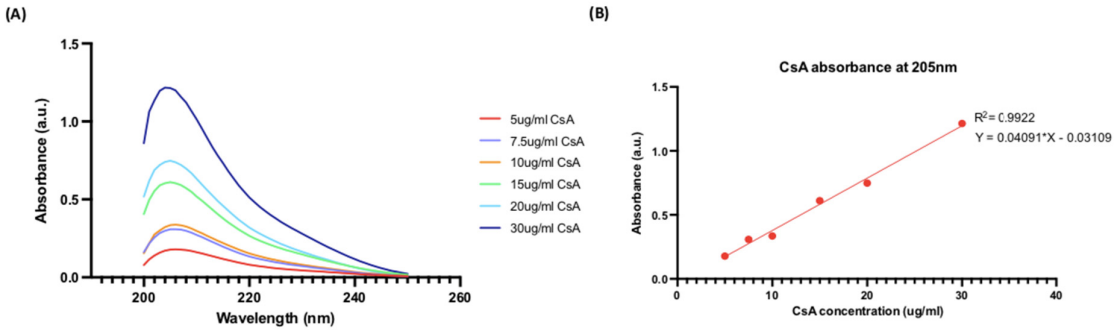

Figure S2. Construction of standard curve for CsA quantification using UV-vis spectrometry in 90% ethanol. (A) UV spectra of various concentrations of CsA. (B) Calibration curve of CsA at various concentrations based on the absorbance at 205 nm. n=1.

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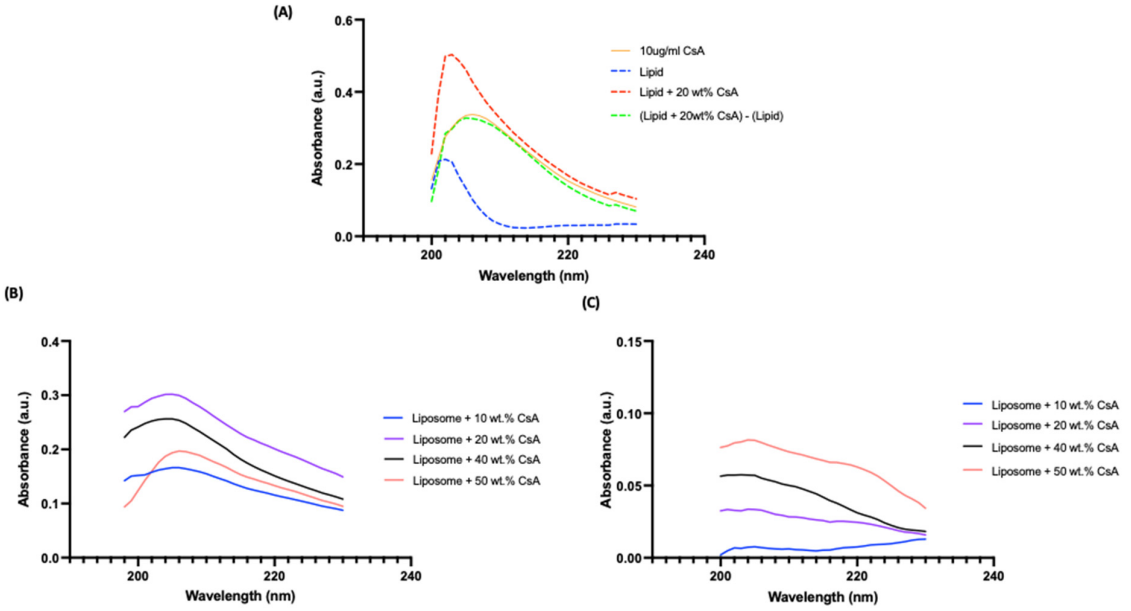

Figure S3. UV spectrum of total CsA and free CsA in samples. (A) UV absorbance of free CsA, lipid molecules, and drug loaded CsA. (B) UV absorbance of total CsA in samples after normalization with lipid molecules. (C) UV absorbance of unloaded CsA in samples after normalization with lipid molecules. n=1.

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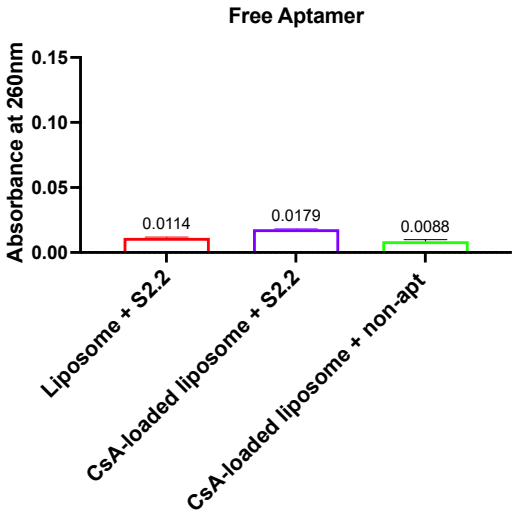

Figure S4. Free aptamer analysis by spectrophotometer at 260 nm and calculations of DNA loading density on liposomes. n=3.

The free aptamer was separated from the liposome by ultracentrifugation at 120,000 rpm for 30 min, and the concentrations of free aptamer were determined using a spectrophotometer at 260 nm. The absorbances of the free aptamer in the aptamer liposome, aptamer CsA liposome, and non-aptamer CsA liposome groups were 0.0114, 0.0179, and 0.0088, respectively. Using the Beer-Lambert Law (path length of 0.05 cm) and the extinction coefficients of S2.2 and non-aptamers (200201 and 272201, respectively), the amounts of free aptamers in a 200 µL reaction were calculated as 166.9 pmol, 262.1 pmol, and 128.8 pmol in the aptamer liposomes, aptamer CsA liposomes, and non-aptamer CsA liposomes, respectively. By subtracting the amount of free aptamers from the initial amount of aptamers (1000 pmol), the approximate amounts of conjugated aptamers were 833 pmol (83% of the initial amount of aptamer), 738 pmol (74%), and 871 pmol (87%) in these groups, respectively.

To estimate the DNA density on liposomes, we calculated that 20 µL of 5.0 mg/mL liposome can adsorb an average of 814 pmol DNA. The molar concentration of phospholipid (5.0 mg/mL) was 6.34 mM, with a total mole of phospholipids at 6.34 µmol. Based on the DLS results, the average particle size of the 20% CsA loading group was 106 nm. Using these values, we estimated that each liposome contains approximately 105,000 phospholipid molecules, assuming the phospholipid head group was 0.6 nm. The effect of cholesterol on expanding or shrinking the average size of the phospholipid head groups was not considered in this estimation. Therefore, the amount of liposome was calculated as 6.34 mM × 20 µL / 105,000 = 1.21 pmol, and each 106 nm liposome contained 673 DNA molecules on average.

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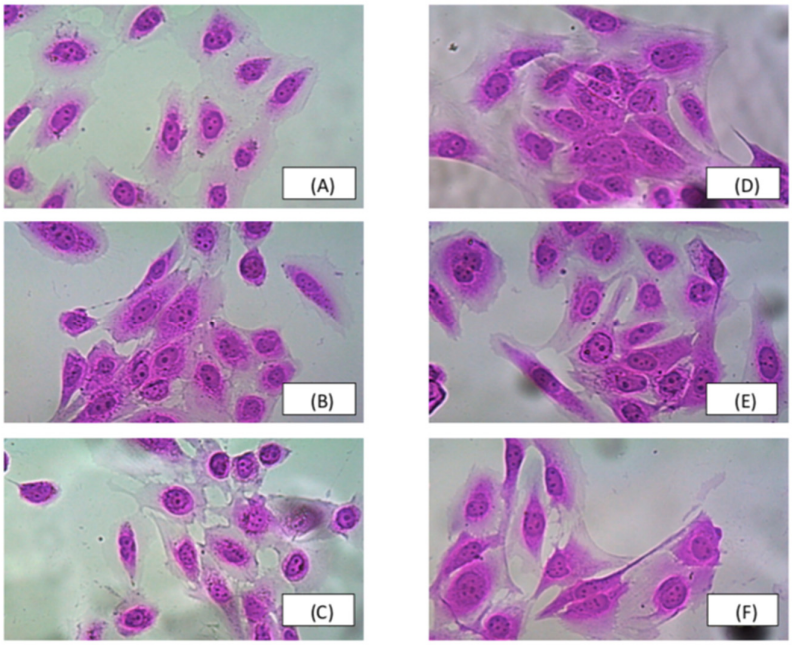

Figure S5. Rose bengal uptake in treated HCECs. (A) Control, (B) DED, (C) 0.001% CsA , (D) 5 µg/mL non-aptamer CsA liposomes, (E) 5 µg/mL aptamer liposomes and (F) 5 µg/mL aptamer CsA liposomes. n=3.

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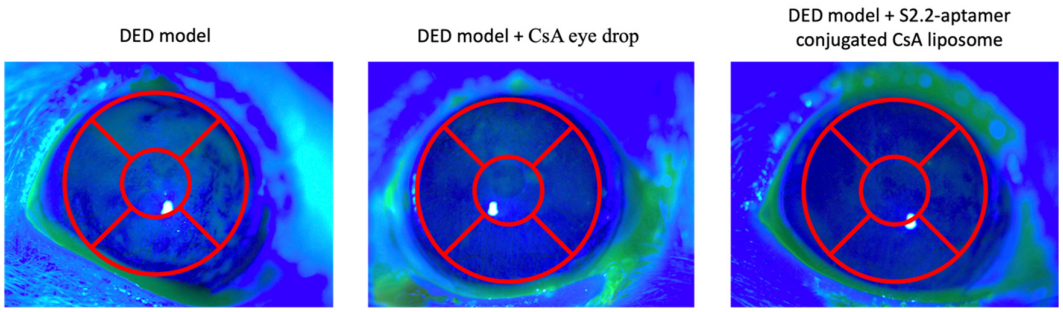
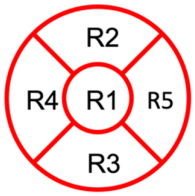
(A)



(B)



(C)

	Estimated mean fluorescein punctate staining score (sum of five regions)
DED model	17.8 ± 0.8
DED model + 0.1%CsA eye drop	11 ± 0.5**
DED model + S2.2-aptamer conjugated CsA liposome	12.5 ± 0.5 **

Figure S6. Evaluation of fluorescein punctate staining score on rat cornea. n=6. Data are shown as mean ± SEM and analyzed using One-Way ANOVA analysis. ** $P < 0.01$ compared with DED group.

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