Dasatinib loaded nanostructured lipid carriers for effective treatment of corneal neovascularization

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S1. Preparation of oligochitosan-NLC

275 mg of the lipid phase (5 mg dasatinib, 112 mg glycerin monosterate, 69 mg Miglyol 812 N and 89 mg Solutol®HS 15) and 10 mL of the aqueous phase (3 mg/mL Gelucire 44/14 and 3 mg/mL soy lecithin) was heated in a water bath of 75 °C. Next, the aqueous phase was dropwise added into the lipid phase under magnetic stirring. 5 min later, the mixed solution was homogenized for 15 min before being rapidly cooled in an ice bath. After string for 10 min in the ice bath, 1 mL of the resulted solution was dropwise added into 1 mL oligochitosan solution (10 mg/mL) at room temperature. Half an hour later, the sample was centrifuged for 5 min at 15000 rpm followed by being filtered by a 0.22 μm filter to remove the unencapsulated drug. Blank and coumarin 6-loaded NLC (coumarin 6-NLC) were prepared in the same manner.

S2. HPLC Method validation

The method was validated based on the guidelines provided by the U.S. Food and Drug Administration (FDA). [1]

Specificity

The specificity of the method was verified by comparing the chromatogram of the blank NLC with that of Dasa-NLC.

Linearity

The calibration samples were freshly prepared and the calibration curves were obtained by plotting the ratio of the peak area of dasatinib versus the concentration of dasatinib. The coefficient of determination (r^2) was calculated. Typically, $r^2 > 0.99$ is

considered to be an acceptable linearity.

Extraction recovery

The recovery of the extraction procedures was determined by the following method. Briefly, different concentrations of dasatinib were spiked into the blank NLC followed by extraction. Next, the concentration was evaluated by the HPLC. The extraction recovery were obtained by calculating the ratio of determined concentration of dasatinib versus the spiked concentration of dasatinib.

Precision

The intra-day precision tests were conducted by analyzing the quality control samples at nominal concentrations (0.6 mg/mL, 0.5 mg/mL and 0.4 mg/mL) with at least six replicates for each concentration within the same day. The inter-day precision was determined on three separate days. The precision should be within ±15% bias and 15% RSD, respectively.

Table S1. Stability observation of NLC and oligochitosan-NLC.

Formulation No.	Soylecithin (mg)	oligochitosan (mg/ml)	Zeta potential (mV)	Size(nm)			
				Day 1	Day 2	Day 3	Day 7
1	0	0	-7.5±0.2	17.80±0.15	Precipitate	-	-
2	20	0	-23.7±-0.8	74.18±0.34	73.79±0.88	Precipitate	-
3	30	0	-28.0±-1.8	80.14±1.95	81.23±0.46	80.04±0.33	80.62±0.71
4	30	10	18.2±0.8	90.23±1.32	91.74±0.31	90.03±0.16	90.87±0.42

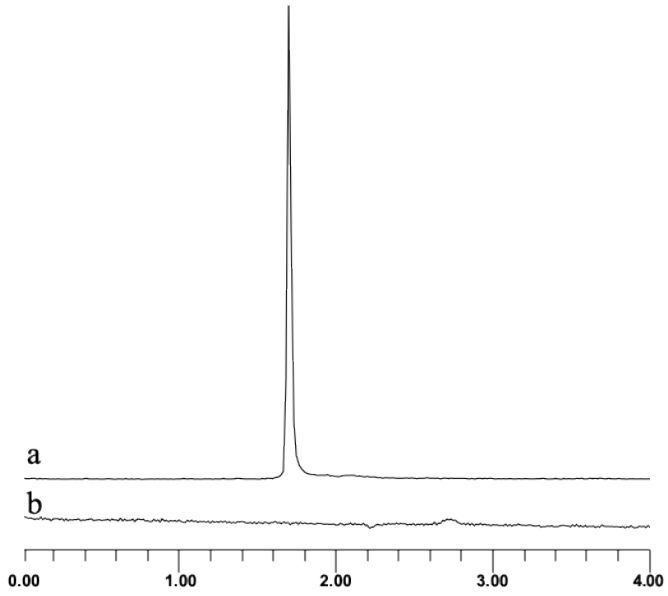

Fig. S1. Chromatograms of dasatinib (a) and blank NLC (b).

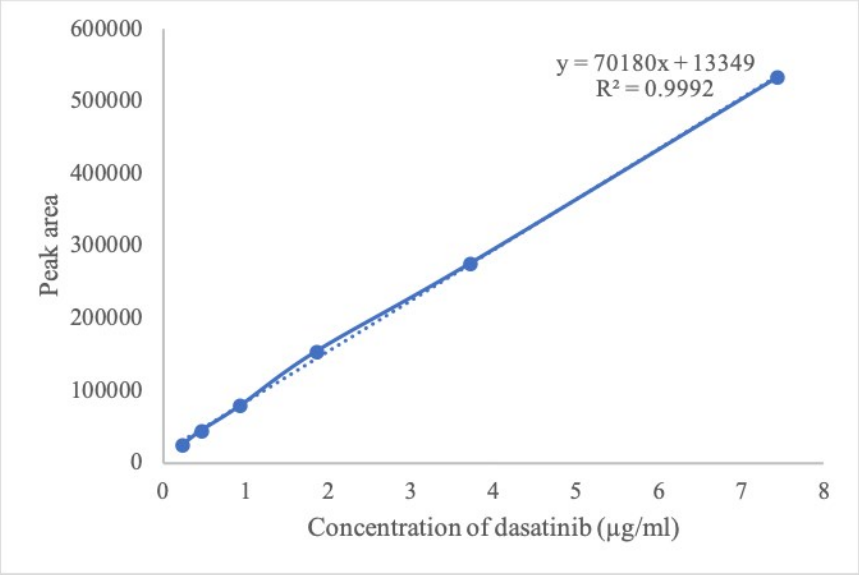

Fig. S2. Standard curve of dasatinib.

Table S2. Extraction recovery of dasatinib in blank NLC (n=5)

Matrix	Spiked concentration （mg/mL）	Detected concentration （mg/mL）	Extraction recovery (%)	RSD
Blank NLC	0.60	0.59±0.02	99.78%	2.78%
	0.50	0.50±0.01	99.11%	2.67%
	0.40	0.39±0.06	97.09%	1.48%

Table S3. Intra-day, inter-day precision of the method (n=6)

Matrix	Spiked concentration (mg/mL)	Intra-day precision (RSD%)	Inter-day precision (RSD%)
Blank NLC	0.60	3.04	6.20
	0.50	3.10	4.88
	0.40	4.55	7.10

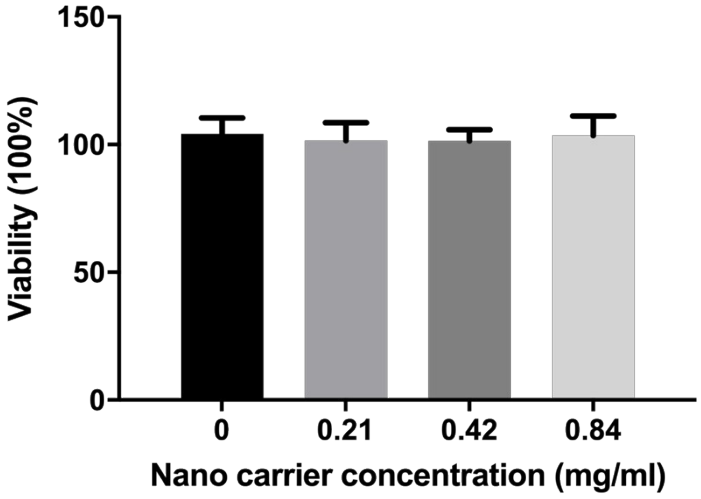

Fig. S3 Cytotoxicity of blank nanoparticles.

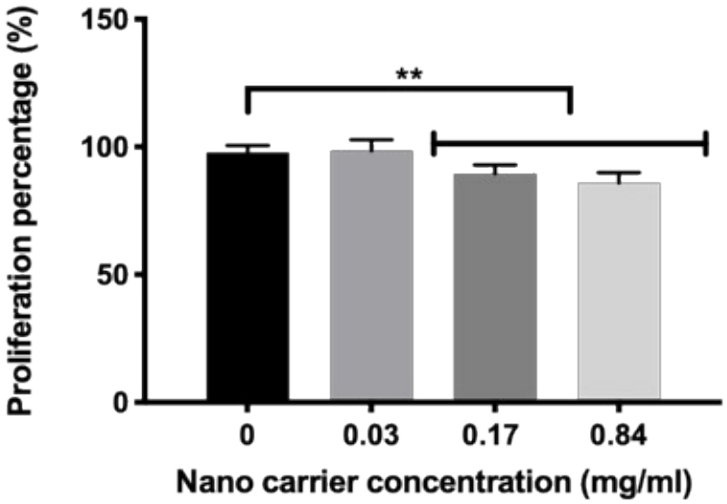

Fig. S4 Anti-proliferation effect of blank nanoparticles.

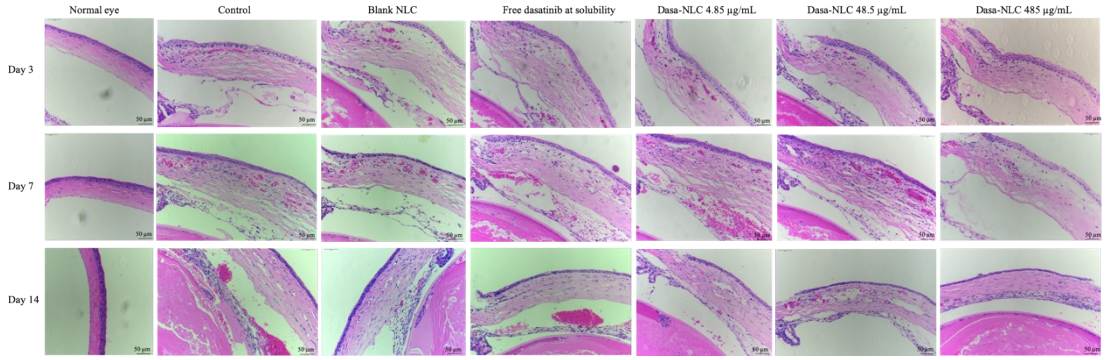

Fig. S5 H&E staining of the peripheral cornea from different groups at different time.

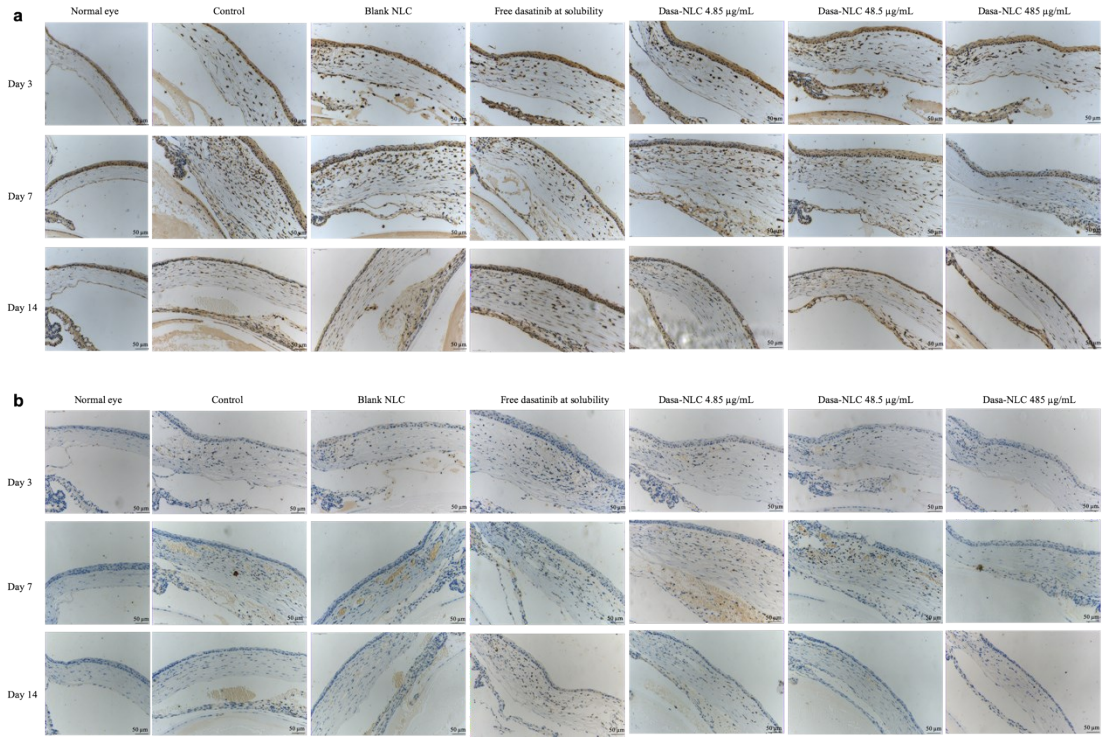

Fig. S6 Immunohistochemistry staining of Src (**a**) and pSrc (**b**) in the peripheral cornea with different treatments at different time.

References

1.Food Administration D., Guidance for industry on bioanalytical method, *Fed Regist*, 2001, **66**, 28526.