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Nanoparticle Mediated Silencing of Tenascin C in Hepatic Stellate Cells: Effect on Inflammatory Gene Expression and Cell Migration

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Electronic Supplementary Information

Methods

The size and shape of the MSNs were visualized using TEM (JEM-1230 TEM) operating at an accelerating voltage of 80 kV. The samples for TEM were prepared by dispersing the MSNs in aqueous solution, and then deposited on a carbon-coated copper grid and air dried for at least 24 h. Particle size distributions were calculated by ImageJ based on a sample of at least 50 particles from different images taken over different quartiles. The size is reported as the average ± SD. The hydrodynamic diameter of the nanoparticles was measured by dynamic light scattering (DLS) using a Malvern Instrument Zetasizer Nano (red laser 633 nm). The equilibration time for each sample was set to 3 min; and three measurements taken on each sample. The DLS measurements were performed on dilute dispersion (0.5 – 1.0 mg/mL) of nanoparticles in 1 mM PBS. ζ-potential dependence on pH was obtained with the same equipment, by measuring the zeta potential in phosphate buffers (0.1M). The organic content was determined by gravimetric analysis (TGA/SDTA851, Mettler Toledo, Columbus, OH). For this experiment, samples were heated from 25 to 800 °C under nitrogen at a rate of 1 °C/min and held at that temperature for 3 h. Protein expression was quantified by scanning densitometry by Quantity One Software (Bio-Rad Laboratories, Hercules, CA) and normalized to GAPDH loading control.

Quantification of Chemically available primary amines using the Kaiser's test

The Kaiser's test was used to quantify primary amines on the surface of MSNs-2 and MSNs. Sodium acetate buffer (2 M, 100 mL) was prepared by dissolving 14.11 g sodium acetate in 86 mL distilled water followed by addition of 14 mL glacial acetic acid (2 M). The pH of the resulting solution was adjusted to 5.4 using HCl. The ninhydrin solution was prepared by dissolving 0.5 g

ninhydrin in 10 mL of ethanol. To carry out the Kaiser's assay; 100 µL of the prepared sodium acetate buffer (pH 5.4) and 70 µL ninhydrin solution were mixed together in a glass vial. Then 10-20 µL of the NP sample was added. The tubes were heated up to 70 °C in an oil bath for 10-15 min. After cooling, 3 mL of ethanol-water mixture in a ratio of 3:2 (vol/vol) was added to each tube. Finally, the absorbance of each solution was measured at 570 nm by a UV-Vis spectrophotometer. The result for each batch is calculated based on the average of four measurements. The final data is presented as the average ± SD for three batches.

Release profile experiment for Alexa-488 labeled DNA duplex loaded MSNs

The experiment was carried out following the next protocol; 0.1 mg of Alexa-488 DNA loaded MSNs (N/P ratio = 10) were dispersed in 1 mL of phosphate buffer solution (pH 7.4), and stirred at 37 °C for 24 h. Samples of 100 µL each were collected at predetermined time points ranging from 0.5 to 24 h. The samples were centrifuged at 13000 rpm for 15 min, and the supernatants were collected for fluorescence analysis at the emission wavelength of 520 nm. A solution of Alexa-488 labeled DNA with the initial concentration of nucleic acids was used as positive control. Fluorescence emission from the phosphate buffer solution at 520 nm was used as background. The data is presented as normalized values based on the initial concentration of Alexa-488 labeled DNA. Results are reported as mean and SD of two independent experiments.

Table S1. Structural properties of MSN materials.

Material	Organic content (% wt.)/TGA	Free amines on NPs (nmol/mg)	Surface Area/BET (m^2/g)	Pore size/BJH (nm)
MSNs-1	7.2	---	785	2.5
MSNs-2	18.5	1617 ± 126	667	2.3
MSNs	38.9	987 ± 150	---	---

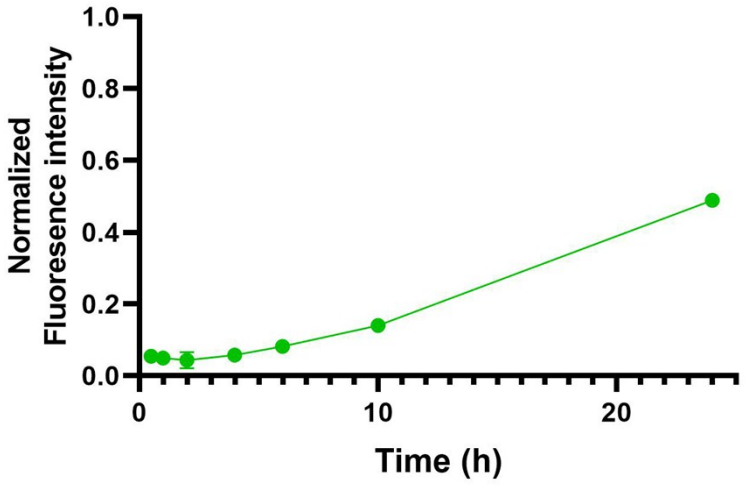

Fig. S1. Release profile of Alexa-488 labeled DNA from MSNs in phosphate buffer solution (pH 7.4) at 37 °C. The data is presented as normalized values based on the initial concentration of Alexa-488 labeled DNA. Results are reported as mean and SD of two independent experiments.

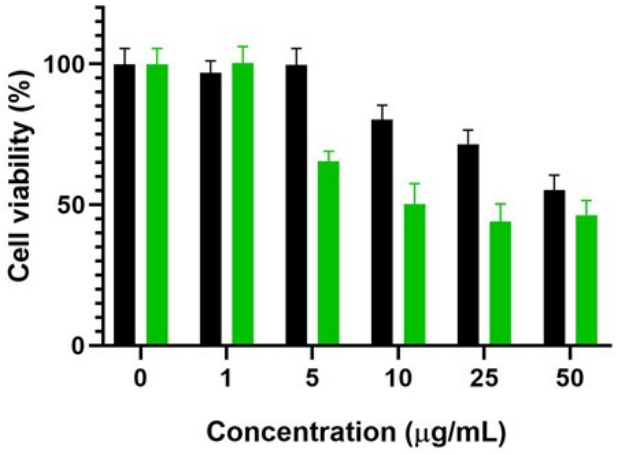

Fig. S2. *In vitro* cytotoxicity of HSCs treated with MSNs (black) and siTnC-MSNs (green). LX2 cells were incubated with MSNs and siTnC-MSNs at various concentrations for 6 h. Data represents mean values for n=3 and error bars indicate SEM.

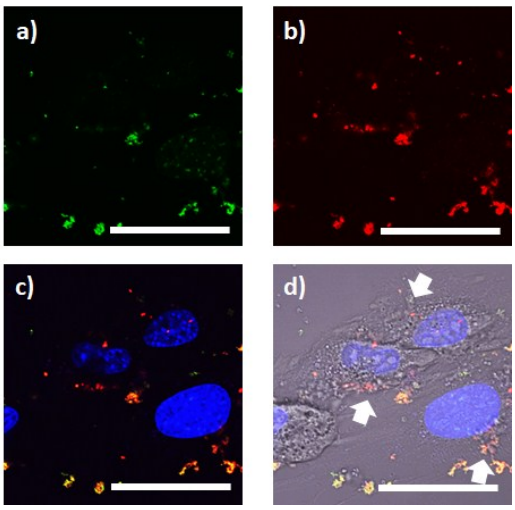

Fig. S3. Internalization of siTnC-MSNs in mouse HSCs was confirmed by confocal microscopy. HSCs were incubated with 5 μg/mL of Alexa647-labeled siTnC-FMSNs for 6 hours and examined by confocal microscopy. MSNs was labelled with FITC and siTnC was labelled with Alexa 647; green denotes fluorescence from FMSNs (**a**); red represents fluorescence from Alexa647-labeled siTnC (**b**); **c** is a composed image where blue and yellow denotes the cell nuclei and the co-localization of Alexa647-labeled siTnC and FMSNs, respectively; and **d** is a composed image of c with the contrast micrograph, the white arrows show the release of siTnC. Scale bar = 20 μm.