



---

# Supplementary Materials: Hepatocellular-Targeted mRNA Delivery Using Functionalized Selenium Nanoparticles In Vitro

Dhireshan Singh and Moganavelli Singh

**Citation:** Singh, D.; Singh, M. Hepatocellular-Targeted mRNA Delivery Using Functionalized Selenium Nanoparticles In Vitro. *Pharmaceutics* **2021**, *13*, 298, <https://doi.org/10.3390/pharmaceutics13030298>

**Publisher's Note:** MDPI stays neutral with regard to jurisdictional claims in published maps and institutional affiliations.



**Copyright:** © 2021 by the authors. Submitted for possible open access publication under the terms and conditions of the Creative Commons Attribution (CC BY) license (<http://creativecommons.org/licenses/by/4.0/>).

1 AUGGAGGAC CCAAGAACAU CAAGAAGGGC CCCGCCCCU UCUACCCCU GGAGGACGGC ACCGCCGGC AGCAGCUGCA CAAGGCAUG AAGCGGUACG  
 101 CCCUGGUGCC CGGCACCAUC GCCUUCACCG ACGCCACAU CGAGUGGAC AUCACCUACG CCGAGUACU CGAGAUAGC GUGCGGUCG CCGAGGCCAU  
 201 GAAGCGGUAC GGCCUGAACA CCAACCACCG GAUCGUGGUG UGCAGCGAGA ACAGCCUGCA GUUCUUAUG CCCGUGCUGG GCGCCUGUU CAUCGGCGUG  
 301 GCCGUGGCC CCGCCAACGA CAUCUACAAC GAGCGGGAGC UGCUGAACAG CAUGGGCAUC AGCCAGCCCA CCGUGGUGU CGUGAGCAAG AAGGGCCUUC  
 401 AGAAGAUCCU GAACGUCGAG AAGAAGCUGC CCAUCAUCCA GAAGAUCAUC AUAUGGACA GCAAGACCGA CUACCAGGGC UUCCAGAGCA UGUACACCUU  
 501 CGUGACCAGC CACCUGCCCC CCGGCUUCA CGAGUACGAC UUCGUGCCCG AGAGCUUCGA CCGGGACAAG ACCAUCGCC UGAUCAUGAA CAGCAGCGGC  
 601 AGCACCGGCC UGCCAAGGG CGUGGCCUUG CCCACCGGA CCGCCUGCGU GCGGUUACG CACGCCGGG ACCCAUCUU CGGCAACAG AUAUCCCG  
 701 ACACCGCAU CCUGAGCGUG GUGCCUUC ACCACGGCUU CGGCAUGUUC ACCACCUUG GCUACCGAU CUGCGGCUUC CCGGUGGUGC UGAUGUACCG  
 801 GUUCGAGGAG GAGCUGUUC UCGGGAGCCU GCAGGACUAC AAGAUCCAGA GCGCCUUGU GGUGCCACC CUGUACGCU UCUUCGCCAA GAGCACCCUG  
 901 AUCGACAAGU ACGACGUGAG CAACCGUCAC GAGAUCCGA GCGGCGGCG CCCCUUGAGC AAGGAGGUG GCGAGGCCU GGCCAAGCGG UUCCACCUUC  
 1001 CCGGAUCCG GCAGGGUAC GGCCUGACC AGACCACCG CGCAUCCUG AUCACCCCG AGGGCGACGA CAAGCCCGG GCCGUGGGA AGGUGGUGCC  
 1101 CUUCUUCGAG GCCAAGGUG UGGACCGGA CACCGCAAG ACCUUGGCG UGAACCAGC GGGCGAGCUG UCGUGCGGG GCCCAUGAU CAUGAGCGGC  
 1201 UACGUGAACA ACCCGAGGC CACCAACGCC CUGAUGACA AGGACGGUC GCUACACAGC GCGCAUCG CCUACUGGA CGAGGACGAG CACUUCUUA  
 1301 UCGUGACCG GCUAAGAGC CUGAUAAGU ACAAGGGCUA CCAGGUGCC CCCGCCGAG UGGAGAGCAU CCUGUCGAG CACCCACA UCUUCGACGC  
 1401 CCGGUGGCC GGCCUCCCG ACGACGACG CCGCGAGCUG CCCGCCCG UGUGGUGUCU GGAGCACGC AAGACCAUGA CCGAGAAGA GAUCGUGGAC  
 1501 UACGUGGCA GCCAGGUGAC CACCGCAAG AAGCUGCGG GCGGCGUGU GUUCGUGAC GAGGUGCCA AGGCCUGAC CGCAAGCUG GACGCCGGA  
 1601 GAUCCGGGA GAUCUGAUC AAGGCCAAG AGGGCGCAA GAUCGCCUG UGA

Figure S1. Sequence of mRNA used in study—proprietary of Trilink BioTechnologies, Inc (San Diego, CA, USA).

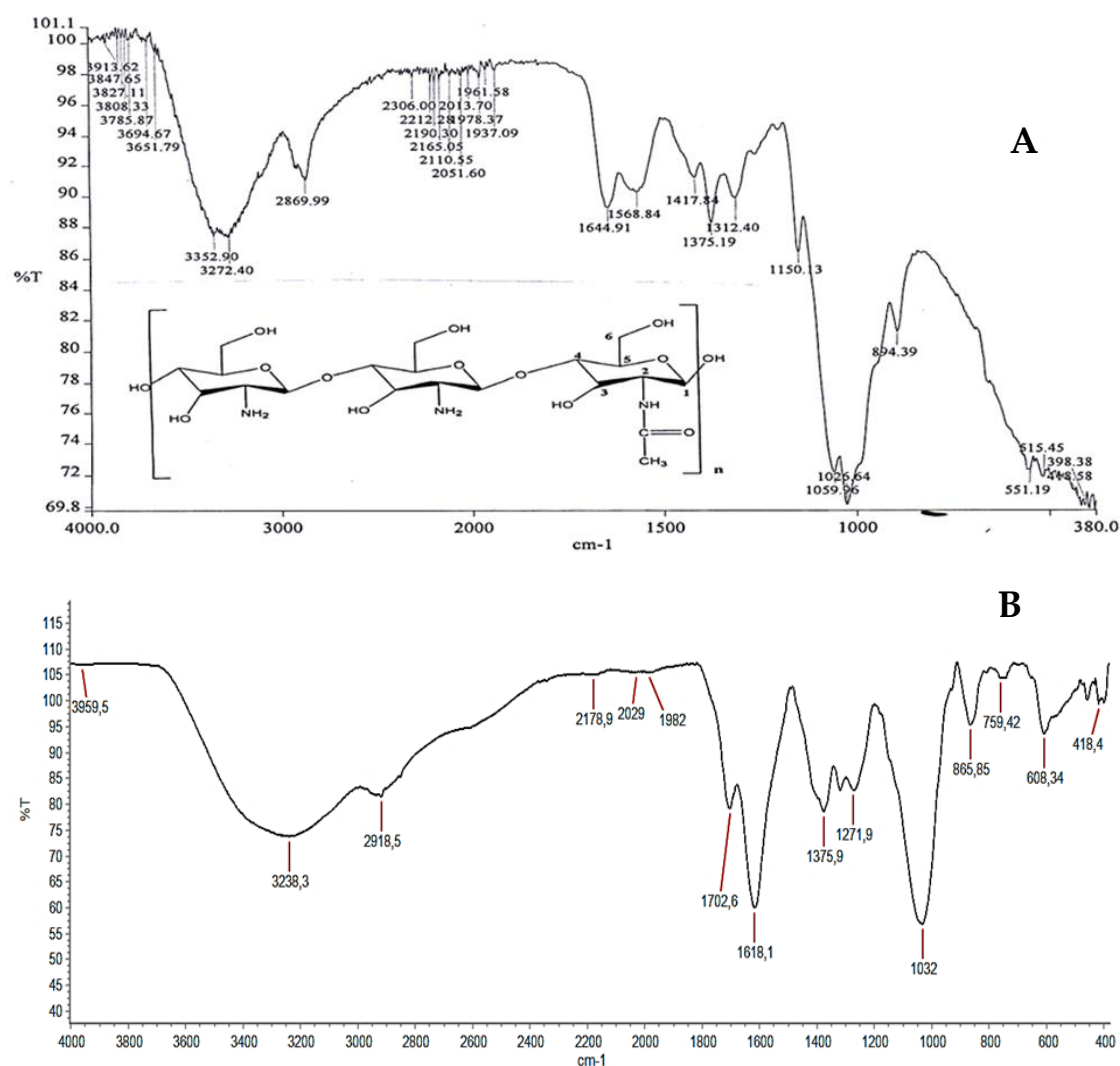


Figure S2. FTIR spectrum of (A) CS and (B) CS-SeNP.

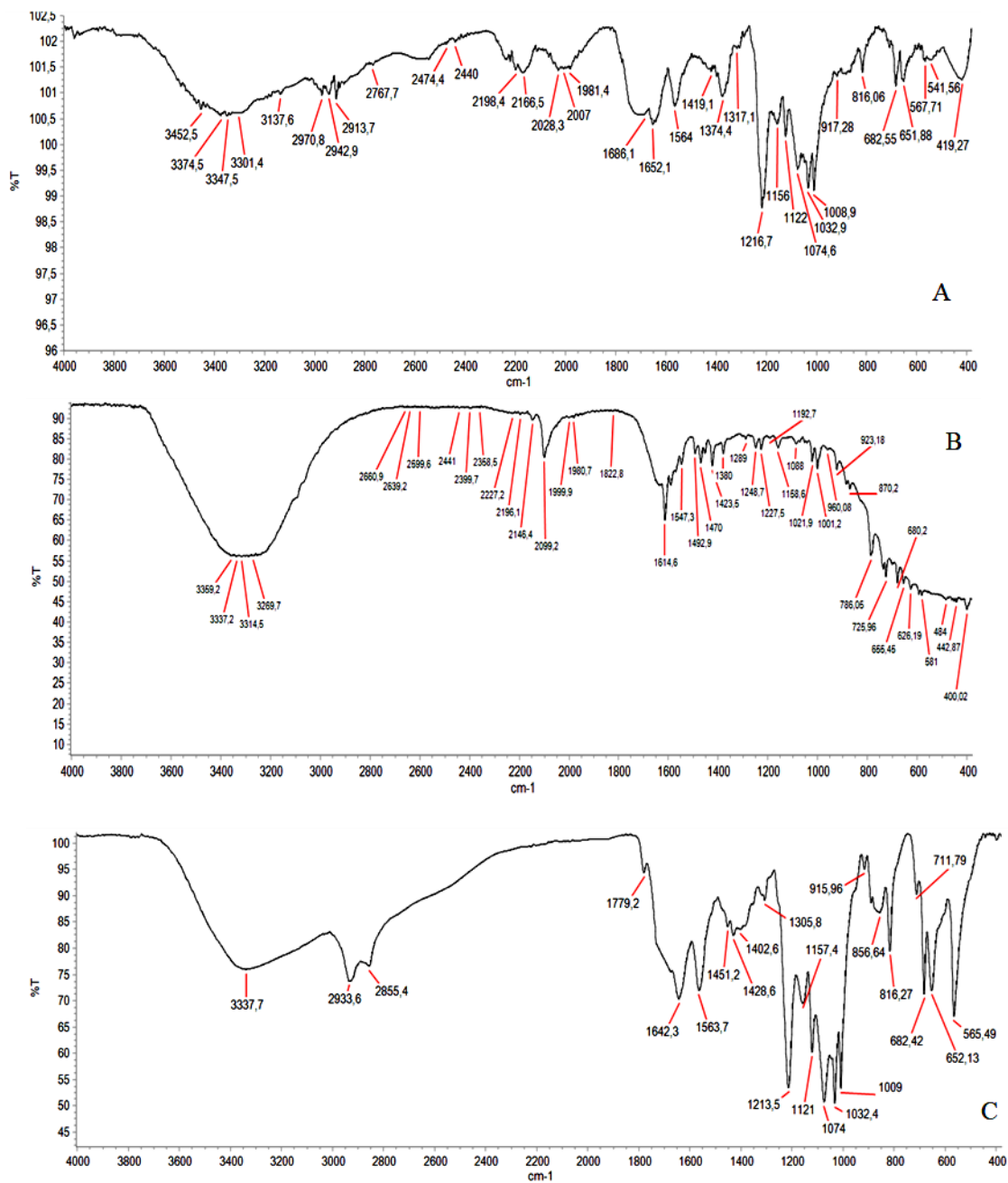
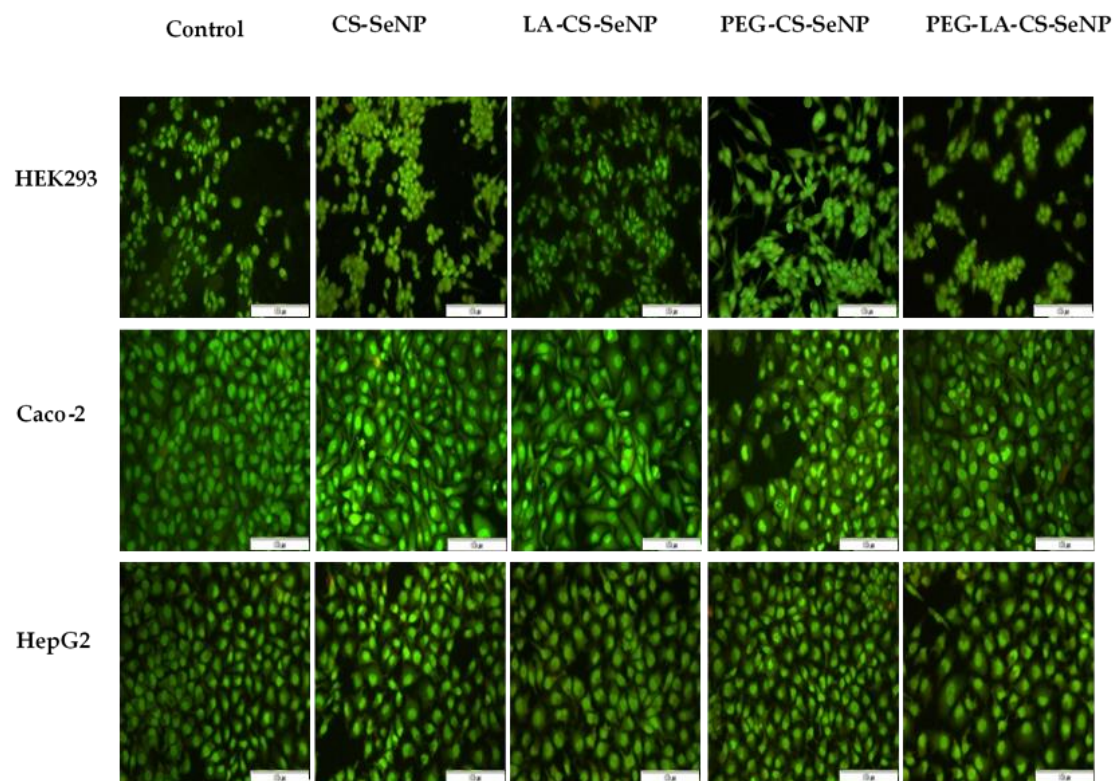


Figure S3. FTIR spectrum (A) LA-CS-SeNP, (B) PEG-CS-SeNP, and (C) PEG-LA-CS-SeNP.



**Figure S4.** Fluorescent images obtained from dual acridine orange/ethidium bromide apoptosis studies after treatment with FSeNPs in the HEK293, Caco-2 and HepG2 cell lines at 20× magnification.

**Table S1.** Sub-optimum, optimum, and supra-optimum functionalized SeNP:mRNA (*w/w*) ratios identified in the band shift assay.

CS-SeNP			
Components	Ratio		
	Sub-optimum	Optimum	Supra-optimum
	1:4.5	1:5	1:5.5
mRNA ( $\mu\text{g}$ )	0.30	0.30	0.30
Nanoparticle ( $\mu\text{g}$ )	1.35	1.51	1.64
LA-CS-SeNP			
Components	Ratio		
	Sub-optimum	Optimum	Supra-optimum
	1:5.5	1:6	1:6.5
mRNA ( $\mu\text{g}$ )	0.30	0.30	0.30
Nanoparticle ( $\mu\text{g}$ )	1.82	1.96	2.10
PEG-CS-SeNP			
Components	Ratio		
	Sub-optimum	Optimum	Supra-optimum
	1:4.5	1:5	1:5.5
mRNA ( $\mu\text{g}$ )	0.30	0.30	0.30
Nanoparticle ( $\mu\text{g}$ )	1.32	1.50	1.65
PEG-LA-CS-SeNP			
Components	Ratio		
	Sub-optimum	Optimum	Supra-optimum
	1:4.5	1:5	1:5.5
mRNA ( $\mu\text{g}$ )	0.30	0.30	0.30
Nanoparticle ( $\mu\text{g}$ )	1.32	1.50	1.65

**Table S2.** Apoptotic indices of FSeNP nanocomplexes in the HEK293, Caco-2, and HepG2 cell lines.

Cell line.	Apoptotic index			
	CS-SeNPs	LA-CS-SeNPs	PEG-CS-SeNPs	LA-PEG-CS-SeNPs
HEK293	0.033	0.012	0.032	0.040
Caco-2	0.032	0.026	0.039	0.015
HepG2	0.035	0.014	0.021	0.031