Design and synthesis of diselenide containing bio-catalyzing polymeric nanocarriers for targeted chemotherapy

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Introduction

- Cancer is a big challenge that has plagued the human beings for ages and one of the most effective treatments is chemotherapy. However, the low tumor-targeting and severe side effects limits the wide clinical application of chemotherapy.
- Microenvironment-targeted therapy strategy could create new opportunities for therapeutic targeting. Stimuli responsive drug delivery systems (DDSs) such as those based on redox, pH, light, enzyme, and ROS sensing deliver chemotherapeutic agents more accurately.
- Redox responsive dual stimuli (pH guide) DDSs work due to differential levels of GSH and pH in tumor verses normal cells.
- The strategy of this delivery could achieve desired results such as no leakage in the blood circulation, tumor-targeting delivery and fast drug release.
- Gelatin protein is a promising drug carrier system because of biodegradable, biocompatible, non toxic nature. Its amphiphilicity can be used to load both hydrophobic and hydrophilic chemotherapeutic drug.

Objective

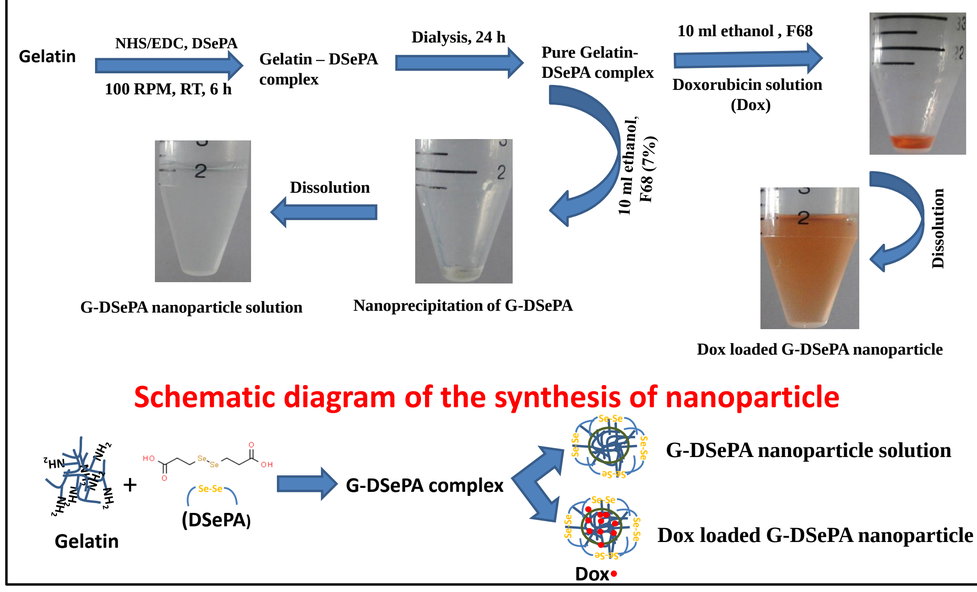
1. Conjugation of diselenodipropionic acid (DSePA) to gelatin surface to use diselenide bond as a bio-catalyzing redox responsive system.
2. Synthesis and characterization of the diselenide bond conjugated gelatin nanoparticle by nanoprecipitation technique.
3. Demonstration of the efficacy of bio-catalyzing dual drug delivery system through *in vitro* release, cytotoxicity and cellular imaging studies.

Instrumentation

- Dynamic Light Scattering (DLS)
- Transmission Electron Microscope (TEM)
- Zeta Potential measurement
- UV-Visible Spectroscopy
- Atomic Absorption Spectroscopy (AAS)

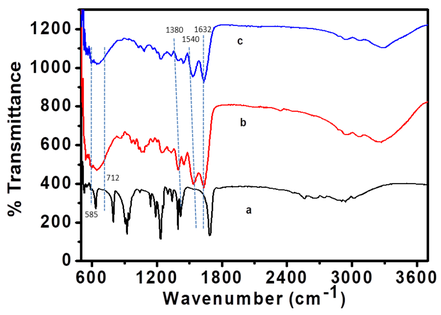
Results and discussion

Synthesis of G-DSePA conjugated gelatin nanoparticle



Schematic diagram of the synthesis of nanoparticle

Characterization of G-DSePA complex



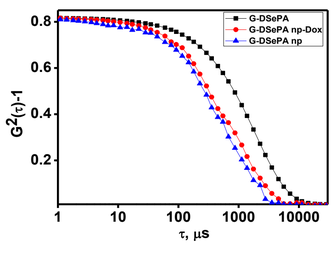
a = DSePA, b = G-DSePA
c = Gelatin (G)

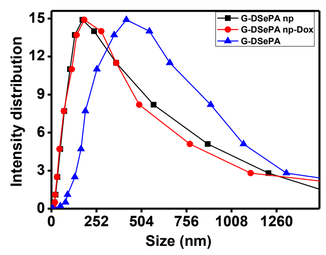
- Reinforcement of peak at 1632, 1540 and 1380 cm^{-1} of G- DSePA confirms the amide bonding between NH_2 group of gelatin and CO_2H group of DSePA.
- Reinforcement of peak in the G- DSePA from 586 to 712 confirms the presence of C-Se bond in the G- DSePA

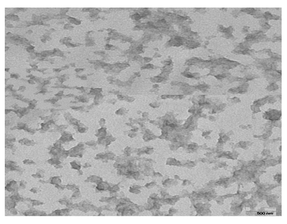
Estimation of selenium

- ✓ Selenium was estimated by atomic absorption spectroscopy
- ✓ 3.4 µg selenium was present in 1 mg gelatin protein

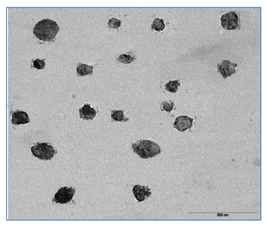
Characterization of gelatin nanoparticle







G-DSePA



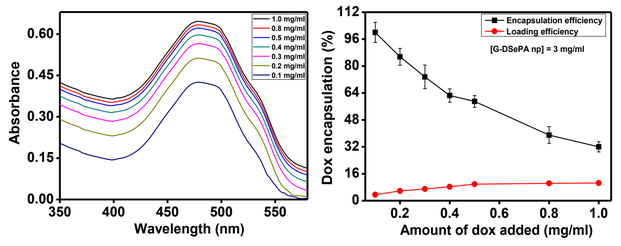
G-DSePA np

Sample	Size (nm) DLS	Size (nm) TEM	Zeta potential (mV)	*Yield (%)**
G-DSePA	424 ± 17	436 ± 18	-11.53 ± 0.59	
G-DSePA np	179 ± 10	181 ± 13	-13.10 ± 0.83	*77*

* *Yield of the nanoparticle was estimated by Bradford protein assay*

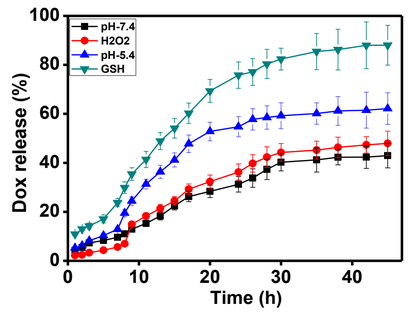
- ✓ Increase in the surface charge in nanoparticle solution over pure G-DSePA solution stabilises nanoparticle in the suspended condition.
- ✓ Morphology of nanoparticle : Spherical shape

Loading of anti cancer drug doxorubicin

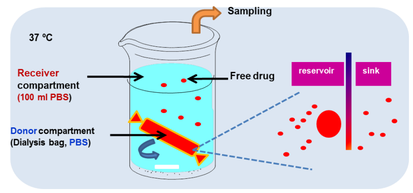


- ✓ Extinction coefficient = 11500 cm^{-1} M^{-1}
- ✓ Encapsulation and loading efficiency were 97% and 10.53% respectively

In vitro drug release study



Time (h)	Release (%) Normal cell condition*	Cancer cell conditions# pH 5.4	Cancer cell conditions# 10 mM GSH
5	7.72	10.64	16.98
20	28.27	53.10	69.53
40	42.12	61.47	87.03

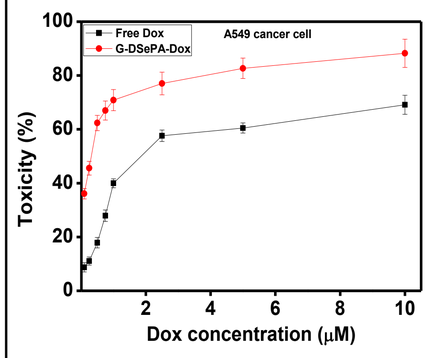


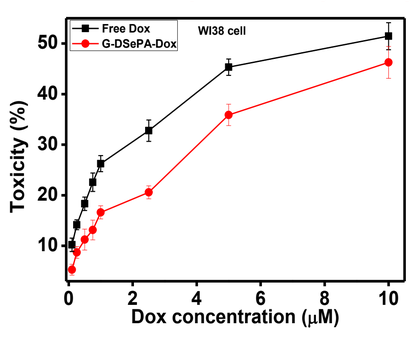
Normal cell condition* → pH 7.4 PBS buffer, temperature 37°c

Cancer cell conditions# → pH 5.4 PBS buffer (acidic), 10 mM GSH (reducing), temperature 37°c

- ✓ The release profile of Dox from G-DSePA nanoparticles was investigated under reservoir-sink condition.
- ✓ Higher drug release at acidic medium facilitates cancer cell specific drug release.
- ✓ At reducing environment cleaving of diselenide bond facilitates higher drug release.

In vitro cytotoxicity study





Normal cell (WI38 cell)		Cancer cell (A549 cell)	
Free Dox	G-DSePA-Dox	Free Dox	G-DSePA-Dox
~10 µM*	> 10 µM*	1.85 µM*	0.32 µM*

* *IC 50 value*

Technique: MTT method
IC 50 value ~ 6 times higher in formulation than free Dox in cancer cell

Technique: Fluorescence microscopy image

Dox concentration: 1 µM
Duration: 6 hrs

Cell lines: A549 lung cancer & WI38 normal lung cell
Nuclear staining dye: DAPI (4',6-diamidino-2-phenylindole)

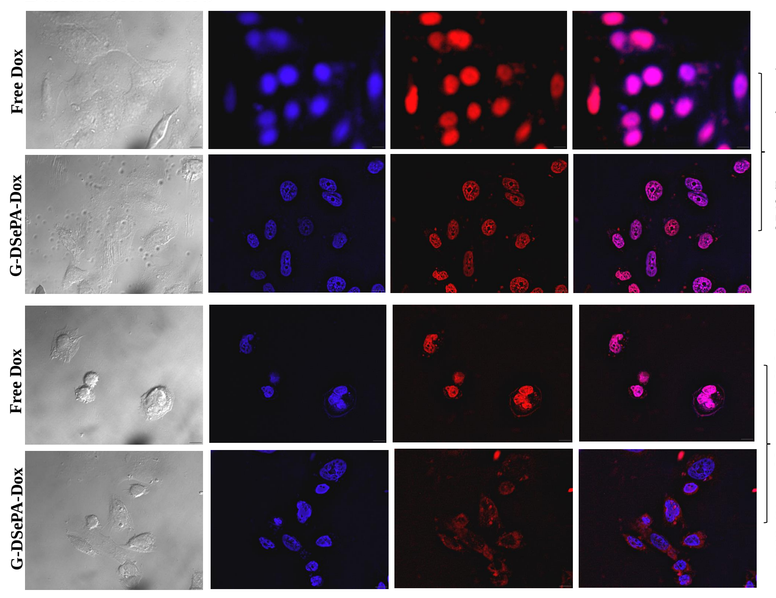
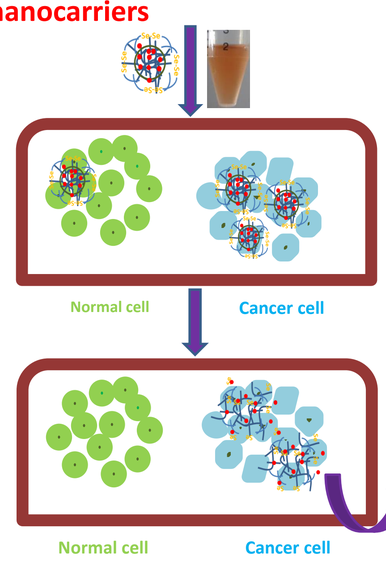
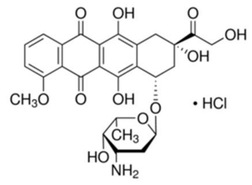


Illustration of dual stimuli responsive G-DSePA-Dox nanocarriers





- ✓ Higher Dox intensity of G-DSePA-Dox nanoformulation in cancer cell compared to normal cell confirms higher Dox uptake in cancer cell.
- ✓ Accumulation of Dox in G-DSePA-Dox nanoformulation occurs at nucleus in cancer cell, this confirms redox sensitive drug release behaviour.

Conclusions

- ✓ Developed DSePA conjugated redox responsive gelatin nanoformulation with ~ 180 nm size and spherical shape for doxorubicin delivery system.
- ✓ Present study concluded higher drug release under tumor microenvironment conditions (e.g. high level GSH and acidic pH) compared to normal cell condition.
- ✓ Our future aim is to study this nanoformulation in *in vivo* mice model.

1. Du J, Choi B, Liu Y, Feng A and Thang SH, Degradable pH and redox dual responsive nanoparticles for efficient covalent drug delivery, Polym. Chem., 2019,10, 1291-1298.
2. Xu C, Song R, Lu P, Chen JC, Zhou YQ, Shen G, Jiang MJ and Zhang W, pH-triggered charge-reversal and redox-sensitive drug release polymer micelles co-deliver doxorubicin and triptolide for prostate tumor therapy, Int. J. Nanomedicine, 2018, 13, 7229-7249 .