

## Supplementary Materials

for

# Novel PLGA-based Nanoformulation Decreases Doxorubicin-Induced Cardiotoxicity

### Physicochemical characterization of nanoformulations

Nanoformulations of lipoDOX and PLGADOX were visualized by transmission electron microscopy (TEM) using the JEM1010 microscope (Jeol, Japan) equipped with a MegaView CCD camera (Olympus, Japan)

. Images were captured using a bright-field mode with an acceleration voltage of 80 kV. The samples were prepared by droplet deposition on a Formvar®-coated copper grid. Dynamic light scattering (DLS) and electrophoretic light scattering (ELS) were performed on the Zetasizer Nano ZS (Malvern Instruments, Malvern, UK) to evaluate the hydrodynamic diameter ( $d_H$ ) and zeta ( $\zeta$ ) potential values, respectively.  $d_H$  was measured using a green laser (532 nm), at a detection angle of 173°. 10 measurements were performed to obtain average  $d_H$  by the intensity distribution function.  $\zeta$  potential was determined from the measured electrophoretic mobility using the Henry equation with Smoluchowski approximation, and the average of 3 measurements is reported. Both DLS and ELS measurements were conducted at 25 °C after a 50-fold dilution of the samples in saline. All data were processed using the Zetasizer software (6.32; Malvern Instruments, Malvern, UK).

**Table S1.** Description of dehydration procedure for rat heart tissues with indicated reagents and times of incubation.

Reagent	Duration
50 % ethanol 1	1 h
50 % ethanol 2	1 h
70 % ethanol	overnight
96 % ethanol 1	1 h
96 % ethanol 2	1 h
100 % ethanol	overnight

**Table S2.** Description of embedding procedure for rat heart tissues with indicated reagents and times of incubation.

Reagent	Duration
Xylen:ethanol (1:1)	1.5 h
Xylen 1	1h
Xylen 2	1h
Paraffin 1	1.5 h
Paraffin 2	1.5 h
Paraffin 3	2 h
Paraffin 4	3 h

**Table S3.** Standard staining procedure for hematoxylin and eosin (H&E).

Reagent	Duration
Xylen 1	5'
Xylen 2	5'
100 % ethanol	2 x 5'
96% ethanol	2'
70% ethanol	2'
Distilled water	2 x 2'
Hematoxylin H	3'
Distilled water	One dip
Running tap water	3'
Distilled water	One dip
Eosin 1%	2'
Distilled water	2'
70% ethanol	2'
96% ethanol	2'
100 % ethanol	2'
Xylen 3	2'
Xylen 4	2'
BioMount	

