PEGylated cationic nanocarriers based on triblock copolymers to combine siRNA therapeutics with anticancer drugs

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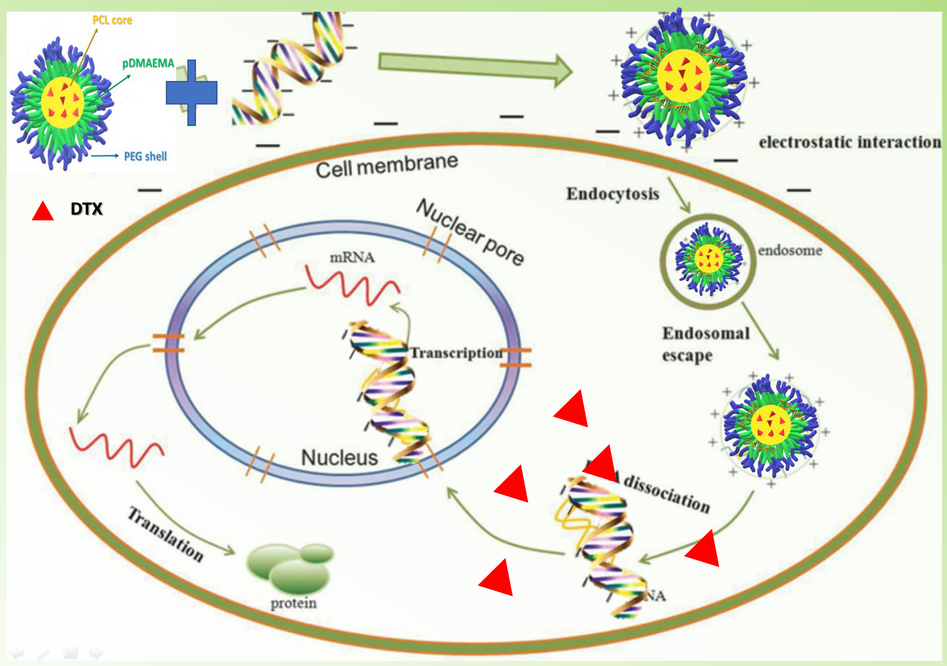
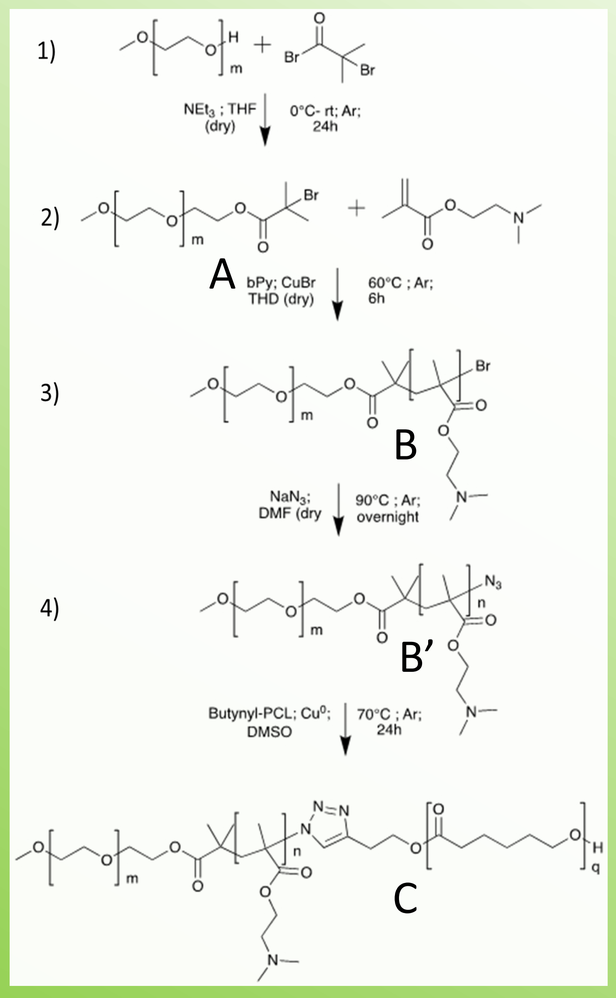
Introduction

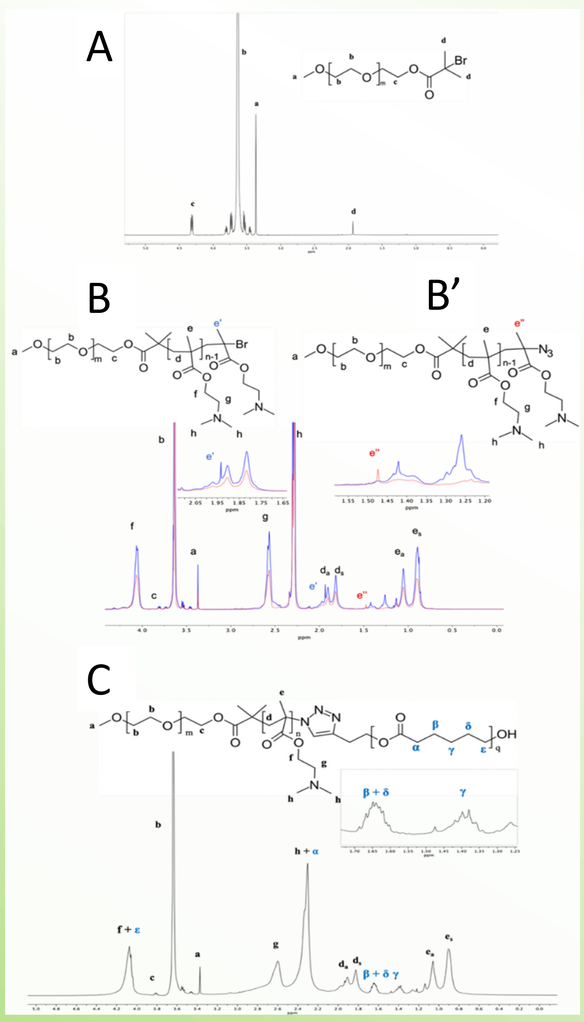
Small interfering RNAs (siRNAs) are emerging as innovative nucleic acid medicines for the treatment of incurable diseases such as cancers. Clinical administration of siRNA therapeutics is still challenging due to the need of safe and efficient delivery carriers. Herein, we propose novel biodegradable NPs based on poly(ethylene glycol) (PEG)- poly(2-dimethyl(aminoethyl) methacrylate) (pDMAEMA)- polycaprolactone (PCL) triblock copolymers with different lengths of the blocks and hydrophilic/lipophilic balance to deliver siRNA alone or in association with a conventional anticancer drug for a potential synergic anticancer therapy. Copolymers were synthesized by a combination of chemical methodologies and characterized by NMR analysis, Fourier Transform Infrared (FTIR) spectroscopy, Gel Permeation Chromatography (GPC) and Differential Scanning Calorimetry (DSC). Copolymers were then employed to prepare NPs through nanoprecipitation. NPs were complexed with a therapeutic siRNA against βIII-tubulin, involved in Multi Drug Resistance mechanisms of different anticancer drugs (TUB-siRNA), and loaded with Docetaxel (DTX). Colloidal properties at pH 7.4 (cytoplasmic pH) and 5.5 (endosomal pH) and buffer capacity of NPS were assessed. Release studies of DTX and siRNA were performed. The transfection efficiency, cytotoxicity and efficiency in silencing TUB gene expression of NPs was evaluated in human melanoma cells (A375).



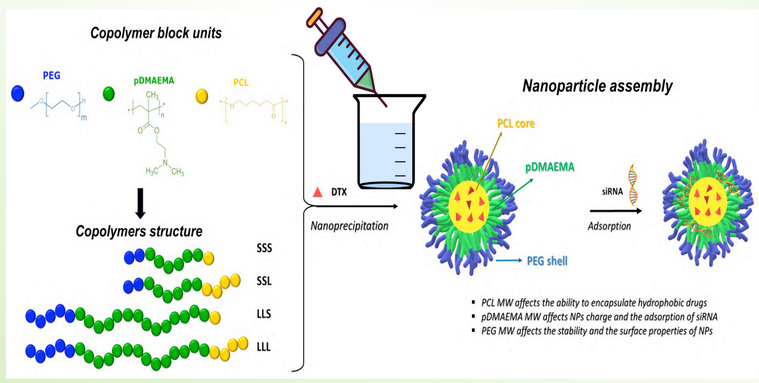


Synthesis of Block Copolymers

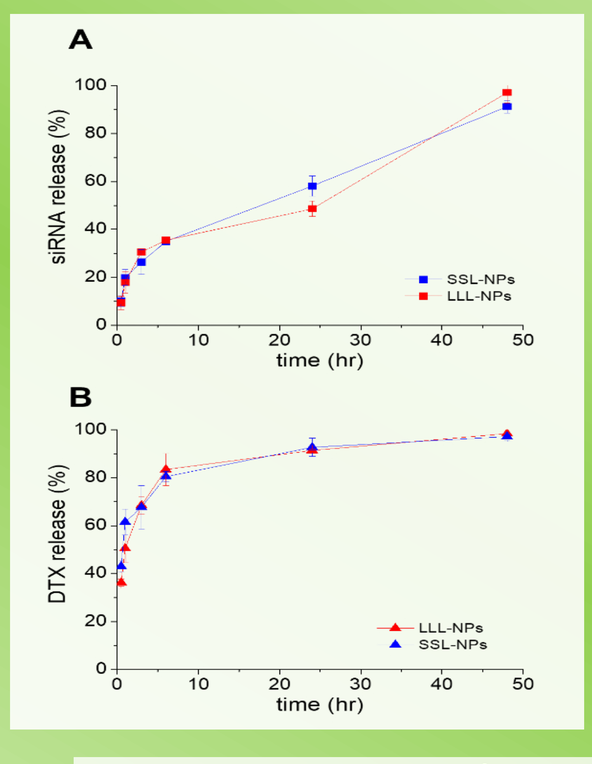
In a first step, $mPEG_m$ (chosen for its stealth properties) was conjugate with α-bromoisobutyryl bromide to obtain a macroinitiator used for atom tranfer radical polymerization (ATRP) of $pDMAEMA_n$ as hydrophilic cationic block for siRNA complexation. Once diblock copolymer was achieved, terminal Br was substituted with $-N_3$. Azido group was used to conjugate a PCL_p biodegradable hydrophobic block bearing an alkyne through copper(I)-catalyzed Huisgen "click" cycloaddition.

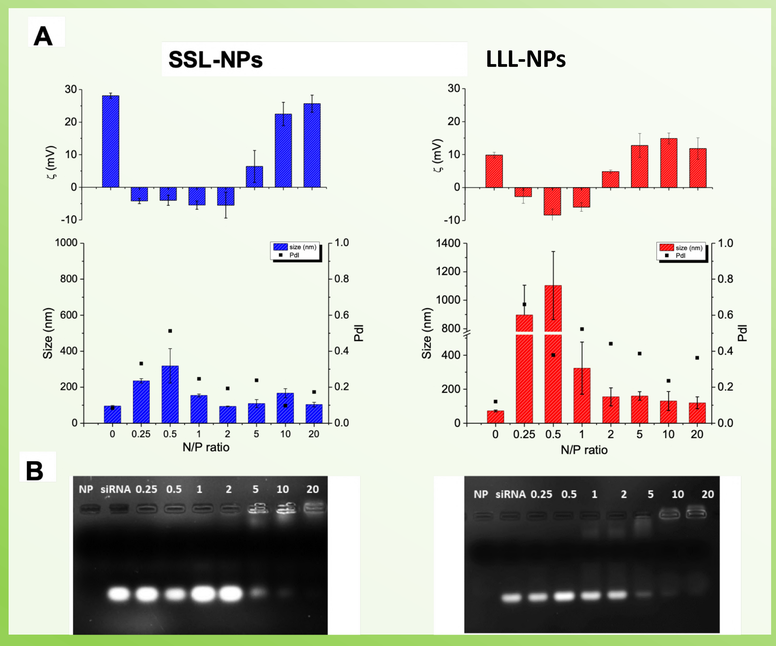


Copolymer	Acronym	Mn (^{1}H NMR)	Mn (GPC)	Mw (GPC)	PDI (GPC)
$mPEG_{2k}pDMAEMA_{5.6k}PCL_{750}$	SSS	8386	8597	11237	1.30
$mPEG_{2k}pDMAEMA_{5.6k}PCL_{4k}$	SSL	11832	11978	16870	1.41
$mPEG_{5k}pDMAEMA_{15k}PCL_{750}$	LLS	20684	20982	25998	1.24
$mPEG_{5k}pDMAEMA_{15k}PCL_{4k}$	LLL	23934	24285	32543	1.34



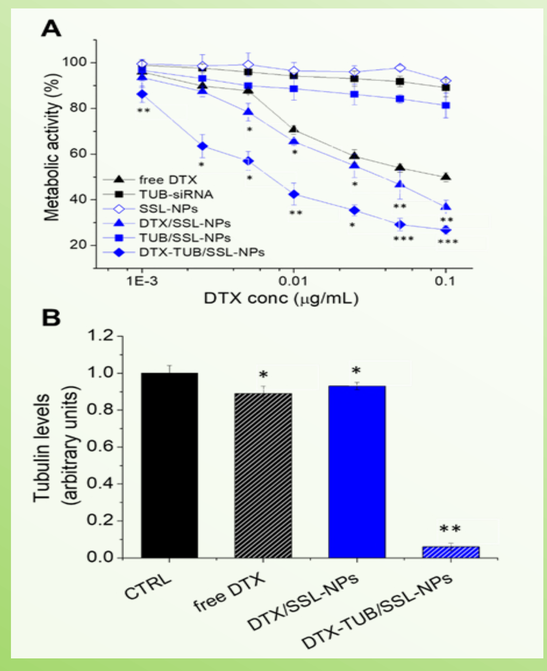
In vitro release profiles of TUB-siRNA (A) and DTX (B) from NPs in PBS 10 mM pH 7.4. Results are expressed as release % over time. Experiments were performed with siRNA/NP complexes at N/P ratio 10.





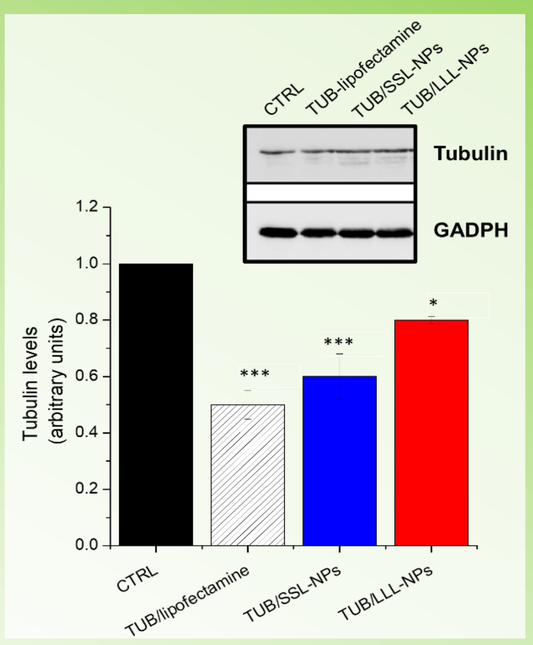
Characterization of NPs complexed with a siRNA Silencer™ Negative Control. (A) Size, polydispersity index and z of siRNA-NPs prepared at different N/P ratios; B) siRNA complexation as evaluated by the gel retardation assay;

Cell metabolic activity of SSL-NPs loaded with DTX, TUB-siRNA at N/P ratio 10 or both drugs toward A375 cells after 48 h of incubation



Quantification of signals of RNA extracted through RT-qPCR by cells after indicated treatments.

In vitro transfection of TUB-siRNA complexed with NPs. A375 cells were treated with SSL- and LLL-NPs complexed with TUB-siRNA at N/P ratio 10 or with TUB-siRNA complexed with Lipofectamine as control for 4 h, and then incubated for 48 h. GAPDH was used as loading control.



Conclusions

Here is reported the development of novel biodegradable NPs based on mPEG-pDMAEMA-PCL triblock copolymers. Through the proper modulation of the copolymer features and its hydrophilic/hydrophobic balance, is possible to achieve core-shell nanostructures able to condense with siRNA with high efficiency and eventually deliver a second anticancer drug in combination. On the basis of the promising results of this research, this system could be deeply investigated in the future in a biological setting in view of a potential synergic anticancer therapy.