Development of self-associating SN-38-conjugated poly(ethylene oxide)-poly(ester) micelles for colorectal cancer therapy

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INTRODUCTION

The main objective of the present work was to develop a polymeric micellar formulation of SN-38 in biodegradable nano-carriers based on poly(ethylene oxide)-poly(ester) block copolymers. For this purpose, conjugation of SN-38 to pendant carboxyl functional groups on methoxy-poly(ethylene oxide)-block-poly(α-carboxyl-ε-caprolactone) (mPEO-*b*-PCCL) or end functional groups on methoxy-poly(ethylene oxide)-block-poly(α-benzyl carboxylate-ε-caprolactone) (mPEO-*b*-PBCL) was pursued (Figure-1). This strategy was expected to enhance the solubilized levels of SN-38 in aqueous media. The results of comparative studies on physicochemical properties, kinetic and thermodynamic stability as well as in vitro cytotoxicity and hemolytic activity of the two generated polymeric micellar formulations, i.e., mPEO-*b*-PCCL/SN-38 and mPEO-*b*-PBCL/SN-38, are presented here.

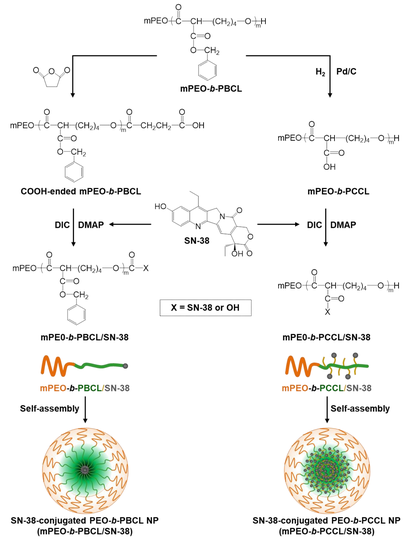

Figure-1: Chemical structures of SN-38, mPEO-*b*-PBCL, mPEO-*b*-PCCL, and schematic procedures to synthesize mPEO-*b*-PBCL/SN-38 and mPEO-*b*-PCCL/SN-38 forming self-assembled micelles.

METHODS

- The polymeric micellar SN-38 conjugates were composed of either methoxy-poly(ethylene oxide)-block-poly(α-benzyl carboxylate-ε-caprolactone) conjugated to SN-38 at the PBCL end (mPEO-*b*-PBCL/SN-38) or mPEO-block-poly(α-carboxyl-ε-caprolactone) attached to SN-38 from the pendent free carboxyl site (mPEO-*b*-PCCL/SN-38).

METHODS

- The chemical structure of block copolymers was confirmed by ^{1}H NMR.
- The physicochemical characterizations of their self-assembled structures including size, surface charge, polydispersity, critical micellar concentration, conjugation content and efficiency, morphology, kinetic stability as well as *in vitro* release of SN-38 were compared between the two formulations.
- *In vitro* anticancer activities were evaluated by measuring cellular cytotoxicity and caspase activation by MTS and caspase-glo 3/7 assays, respectively.
- The hemolytic activity of both micellar structures against rat red blood cells was also measured.

RESULTS

Table-1: Physicochemical characteristics of the self-assembled block copolymers and SN-38-conjugated block copolymer micelles (n = 4).

Micellar Formulations [a]	Size [b] ± SD (nm)	PDI [c] ± SD	Zeta Potential [d] ± SD (mV)	CMC [e] ± SD (μgmL^{-1})	SN-38 Loading [f] (% w/w)
$mPEO_{114}$-b-$PBCL_{12}$	46.25±0.11	0.12±0.01	0.09±0.03	4.43±0.21	-
$mPEO_{114}$-b-$PBCL_{12}$/SN-38	43.60±0.14 [g]	0.13±0.01	-1.14±0.23 [g]	3.88±0.11 [g]	11.47±0.10
$mPEO_{114}$-b-$PCCL_{20}$	56.76±0.41	0.17±0.01	0.04± 0.01	69.92±0.82	-
$mPEO_{114}$-b-$PCCL_{20}$/SN-38	38.47±0.34 [g]	0.11±0.02	-1.69±0.18 [g]	54.57±0.12 [g]	12.03±0.17

[a] The number shown in the subscript indicates the degree of polymerization of each block as determined by 1H NMR spectroscopy.
[b] Hydrodynamic diameter (Z average) determined by DLS.
[c] Average PDI of micellar size distribution.
[d] Average surface charge (zeta potential) of the micelles.
[e] Average CMC measured by DLS.
[f] SN-38 loading (w/w %) = {(Amount of conjugated SN−38)/(Total amount of polymer)} ×100; measured using UV-Vis spectroscopy.
[g] Differences were considered significant if *p ≤ 0.05, **p ≤ 0.01, ***p ≤ 0.001, or ****p ≤ 0.0001 following unpaired student's t test when compared to their counterpart polymeric micelles without SN-38.

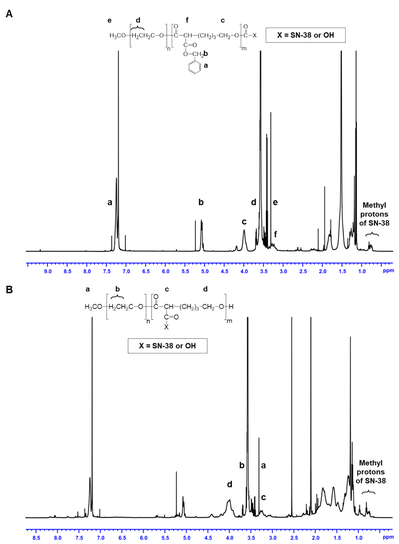

Figure-2: ^{1}H NMR spectra and corresponding peak assignments for (A) mPEO-*b*-PBCL/SN-38, (B) mPEO-*b*-PCCL/SN-38.

RESULTS

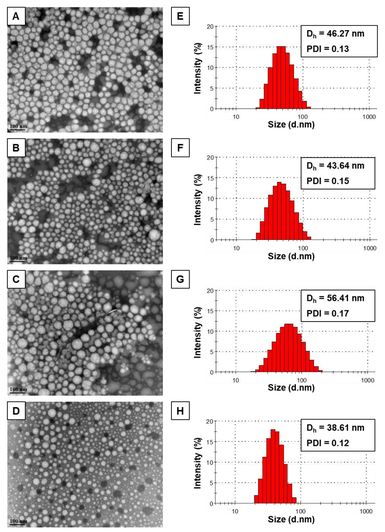

Figure-3: TEM images of (A) mPEO-*b*-PBCL, (B) mPEO-*b*-PBCL/SN-38, (C) mPEO-*b*-PCCL, and (D) mPEO-*b*-PCCL/SN-38 micelles. The bar in the bottom left corner of each image indicates a scale of 100 nm. Hydrodynamic diameter (Dh), PDI, and size distribution of (E) mPEO-*b*-PBCL, (F) mPEO-*b*-PBCL/SN-38, (G) mPEO-*b*-PCCL, and (H) mPEO-*b*-PCCL/SN-38 micelles in aqueous medium were obtained using dynamic light scattering (DLS).

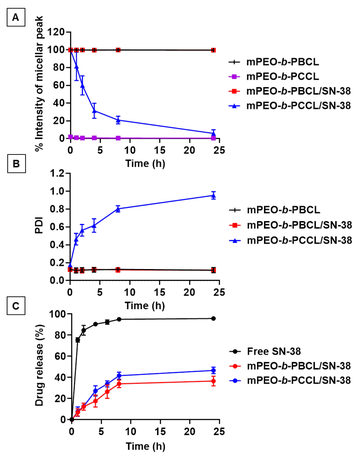

Figure-4: Average (A) percentage of intensity and (B) PDI of mPEO-*b*-PBCL, mPEO-*b*-PBCL/SN-38, and mPEO-*b*-PCCL/SN-38 micellar peak (3 $mgmL^{-1}$) in the presence of SDS (20 $mgmL^{-1}$) as a function of time up to 24 h. (C) The drug release profile of mPEO-*b*-PBCL/SN-38 and mPEO-*b*-PCCL /SN-38 micelles compared to free SN-38 in 4% albumin in ultrapure water at 37ºC. Data are represented as mean ± SD (n = 3).

Table-2: Calculated difference factor (f1) and similarity factor (f2) for SN-38 release profiles from mPEO-*b*-PBCL/SN-38 and mPEO-*b*-PCCL/SN-38 micellar formulations. The profiles were considered similar if f1 ≤ 15 and f2 ≥ 50.

Formulations	Difference factor (f_1)	Similarity factor (f_2)
Free SN-38 and $mPEO_{114}$-b-$PBCL_{12}$	75.01	8.73
Free SN-38 and $mPEO_{114}$-b-$PCCL_{20}$/SN-38	68.27	10.65
$mPEO_{114}$-b-$PBCL_{12}$/SN-38 and $mPEO_{114}$-b-$PCCL_{20}$/SN-38	26.95	56.97

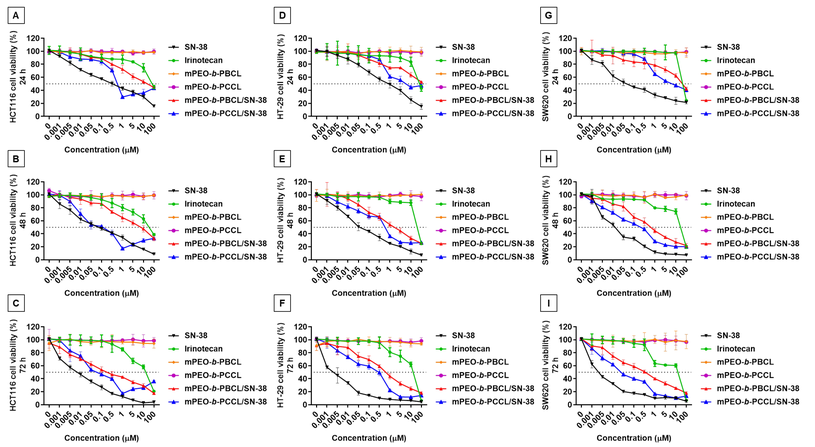

Figure-5: *In vitro* cytotoxicity assay for free SN-38 (black), irinotecan (green), mPEO-*b*-PBCL (orange), mPEO-*b*-PCCL (purple), mPEO-*b*-PBCL/SN-38 (red), and mPEO-*b*-PCCL/SN-38 (blue) in (A-C) HCT116, (D-F) HT-29, and (G-I) SW620 cell lines after 24 h, 48 h, and 72 h incubation at 37ºC in 5% CO_2. The cells were treated with the free drugs and polymeric micelles with a range of concentration from 0.001 μM to 100 μM. SN-38 was solubilized with DMSO and untreated cells received only 0.1% DMSO. Each point represents mean ± SD (n = 4).

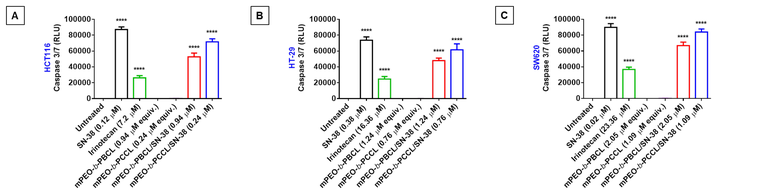

Figure-6: Caspase activity assay for free SN-38, irinotecan, mPEO-*b*-PBCL, mPEO-*b*-PCCL, mPEO-*b*-PBCL/SN-38, and mPEO-*b*-PCCL/SN-38 in (A) HCT116, (B) HT-29, and (C) SW620 cell lines. The cells were treated with the media containing the respective IC_{50} (24 h) concentrations of free SN-38, irinotecan, mPEO-*b*-PBCL/SN-38, and mPEO-*b*-PCCL/SN-38 for 6 h. Data are expressed as mean ± SD (n = 6).

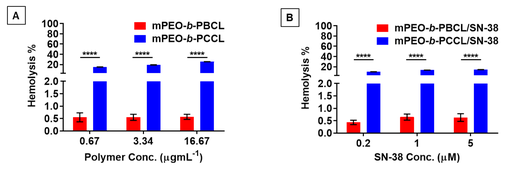

Figure-7: Hemolytic activity of (A) mPEO-*b*-PBCL and mPEO-*b*-PCCL; (B) mPEO-*b*-PBCL/SN-38 and mPEO-*b*-PCCL/SN-38 micellar formulations against rat RBCs. Each error bar represents the mean ± SD (n = 3). Isotonic PBS and full hemolysis by pure water were used as negative and positive controls, respectively.

CONCLUSION

The overall results from this study uphold mPEO-*b*-PBCL/SN-38 over mPEO-*b*-PCCL/SN-38 micellar formulation as an effective delivery system of SN-38 that warrants further preclinical investigation.

REFERENCE & ACKNOWLEDGEMENTS

1. Sadat *et. al.* Pharmaceutics. 2020 Oct 29;12(11):1033. doi: 10.3390/pharmaceutics12111033

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