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The effect of Amine modification on siRNA delivery of redox-responsive PEGylated amphiphilic micellar nanoparticles for triple negative breast cancer therapy

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SUMMARY

We developed a set of methoxy poly(ethyleneglycol)-co-poly(N3-ε-caprolactone) (mPEG-N_3PCL) copolymers with different types of amine linkers and investigated the contribution of the introduced amino linker to the gene delivery efficiency of nanoparticles. The nanoparticles were crosslinked from the caprolactone regions with a redox-responsive linker. Formulation variables including Redox-crosslinking and N:P ratio was examined to obtain nanoparticles with optimal size and highest siRNA entrapment efficacy (EE). The nanoparticles were characterized by DLS, and nanodrop UV-vis spectroscopy. Nanoparticle size, polydispersity index and siRNA entrapment efficacy were found to depend on the all the examined formulation variables specially the N:P ratio. The formulation with N:P ratio of 5 with 56.13 nm in size and 92.24% EE was chosen as the optimal formulation. Controllable size, loading efficiency and release pattern by redox-responsivity make this class of novel carriers a promising candidate for tumour targeted gene delivery applications.

INTRODUCTION

Triple negative breast cancer (TNBC) has gained more and more attention because of its high mitotic indices, aggressive progression, poor prognosis, lack of standard therapies and low overall survival rates [Zhang et al., 2021]. Due to the very limited and low efficient treatment options for TNBC, recent studies have shifted toward new generations of anti-cancer agents. Among those, small interfering RNA (siRNA) gene therapy has been considered as a promising alternative approach because it can potently suppress the expression of important chemotherapy resistance inducer genes in TNBC [Wan et al., 2021]. However, the development of efficient siRNA delivery systems appears to be crucial due to the poor stability, rapid clearance and poor cellular uptake of siRNA's [Chadar & Kesharwani, 2021].

One of the important therapeutic targets in TNBC is glutathione (GSH), thus redox-responsive nanocarriers is a promising route for developing controlled site-specific TNBC therapies. The enhanced level of GSH in tumour microenvironment triggers the release of payload from the redox-responsive nanocarriers. These types of nanocarriers can be developed by using crosslinker containing a disulfide bond which will be cleaved by GSH after the uptake of particles by cancer cells, and release the drug/gene cargo in a triggered manner for maximum efficacy [Mollazadeh et al., 2021].

MATERIALS AND METHODS

Herein, we developed an amine modified mPEG-N3PCL copolymers via click chemistry with controllable molar content of amine linkers, and investigated the contribution of the introduced amino linker to the gene entrapment efficiency and size of micellar nanoparticles. The nanoparticles were prepared via nanoprecipitation method and crosslinked from the caprolactone regions with a redox-responsive linker. Formulation optimization

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[Moradi et al, 2021] was performed by changing the variables, Redox-crosslinking and N:P ratio to obtain nanoparticles with optimal size and highest siRNA entrapment efficacy (EE). The nanoparticles were characterized by dynamic light scattering (DLS) for size and polydispersity index measurement and nanodrop UV-vis spectroscopy for siRNA entrapment efficacy.

RESULTS AND DISCUSSION

Nanoparticle size and siRNA entrapment efficacy were found to depend on both N:P ratio and crosslinking. Increasing N:P ratio resulted in the significant enhancement of entrapment efficacy and size reduction due to the stronger electrostatic interaction between amine modified polymer and siRNA molecules. Furthermore, the formulations with primary amine linker formed smaller size micelles with higher siRNA entrapment compared to the ones with tertiary amine linker, which confirms the stronger polarity of primary amine groups and their better interaction with siRNA molecules (Table 1).

Table 1. *Size, Polydispersity index and siRNA entrapment efficacy of mPEG-PCl micellar nanoparticles*

Sample Code	Linker Type	N:P ratio	Polymer: RR-Crosslinker molar ratio	Z-Average (nm)	PDI	Entrapment Efficacy (%)
1a	Tertiary Amine	1	-	147.8	0.25	23.58
1b	Tertiary Amine	5	-	108.0	0.24	41.95
1c	Tertiary Amine	10	-	106.2	0.34	64.62
1d	Tertiary Amine	20	-	84.06	0.48	72.14
2a	Primary Amine	1	-	94.13	0.22	45.8
2b	Primary Amine	5	-	61.44	0.41	92.24
2c	Primary Amine	10	-	56.13	0.27	94.93
2d	Primary Amine	20	-	50. 02	0.25	96.87
2e	Primary Amine	20	1:4	65.81	0.18	95.93

The formulation with primary amine linker and N:P ratio of 5 with 56.13 nm in size and 92.24% EE was chosen as the optimal formulation due to the higher loading efficacy of siRNA. Although, the Redox-responsive crosslinked micelles had larger size than the non-crosslinked formulation, but their ability to have controlled gene release in high glutathione tumour microenvironment makes them more promising for the tumour targeted gene delivery.

CONCLUSIONS

Controllable size, loading efficiency and Redox-responsivity make this class of novel carriers a promising candidate for gene delivery applications.

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