Development of EGFR-Targeted Dual-Loaded Polymeric Nanoparticles for Triple Negative Breast Cancer

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Abstract

Triple-negative breast cancer (TNBC) is a subtype of breast cancer that accounts for 15-20 % of all breast cancer cases [1]. TNBC is extremely challenging to treat using conventional treatment modalities like chemotherapy, radiotherapy, and surgery [2]. Our study aimed to solve the problems of TNBC treatment, such as low efficacy, toxicity, and poor site-specific drug delivery using dual-loaded antibody-targeted nanotherapeutics. We synthesized HEMA-PLA macromonomer, HEMA-PLA-cisplatin, and PEGylated methacrylate-polylactide copolymer containing cisplatin by the ring-opening polymerization, EDC/DMAP coupling, and RAFT polymerization, respectively. The nanoparticles had an average diameter of 162 nm and a negative zeta potential (-12.4 mV). Cetuximab (CTX), a mAb that binds to the EGFR, was attached to the surface of the NPs to enhance its targetability to TNBC. The *in vitro* drug release study showed that both drugs were completely released by 10 days at pH 5. The cell cytotoxicity of CTX-NPs after 96 h of incubation was found to be 2-fold higher than that of other groups (plain NPs and free drugs) by CellTiterGlo assay. The study indicates that CTX-targeted polymeric NPs that contain cisplatin and paclitaxel are effective in treating TNBC [3].

Background

The TNBC lacks receptors such as human epidermal growth factor receptor 2 (HER2), Estrogen receptor (ER), and Progesterone receptor (PR), which are expressed in breast cancers [4]. Although TNBC does not have the most common receptors expressed in breast cancers, it has some receptors, including EGFR (epidermal growth factor receptor) at 45-70%, PD-L1, CTLA-4, and PD-1 [5]. Additionally, drug resistance limits conventional therapies , and for this reason, monotherapy with a single agent has not been successful in the treatment of TNBC.

Figure 1. Difficulties in treating TNBC and the lack of important receptors [6].

Methods

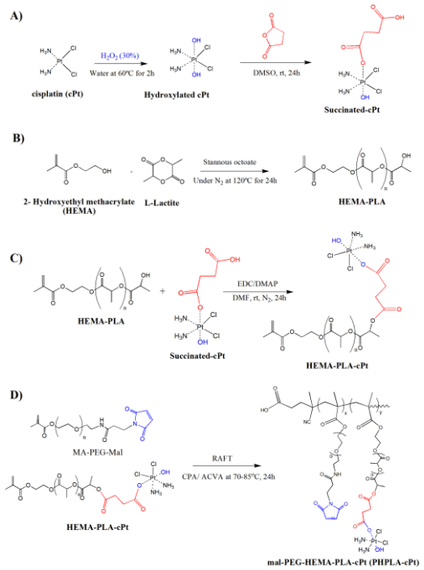

Figure 2. Synthesis scheme of cisplatin –polymer conjugate. A) Succinated cisplatin synthesis from hydroxylated cisplatin and succinic anhydride at 1:1 molar ratio. Hydroxylated cisplatin was obtained by treatment of hydrogen peroxide; B) HEMA–PLA synthesis by ring-opening polymerization of L-lactide with 2-Hedroxyethyl methacrylate at 5.6:1 molar ratio; C) Synthesis of HEMA-PL-cPt conjugate from hydroxyethyl methacrylate polylactide and succinated cisplatin at 1:1 molar ratio using carbodiimde reaction; D) RAFT polymerization of MA-PEG-mal and HEMA-PLA-cPt at 10:1 molar ratio to obtain mal-PEG-HEMA-PLA-cPt (PHPLA-cPt).

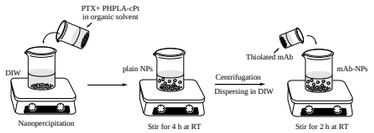

Figure 3. Fabrication of plain- and mAb-nanoparticles (NPs) using nanoprecipitation method and coupling antibody to the NPs.

Methods/Results

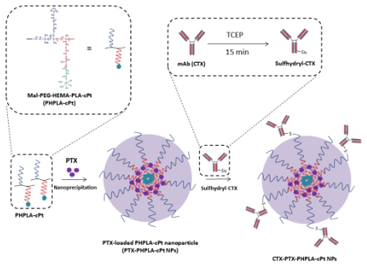

Figure 4. Scheme of preparation of nanoparticles containing PTX and cisplatin and attachment of Cetuximab (CTX) to their surfaces.

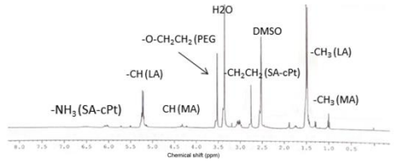

Figure 5. Proton NMR spectra of PHPLA-cPt copolymer. LA represents Lactate, MA represents methacrylate moiety, and SA represents succinic acid residue.

Results

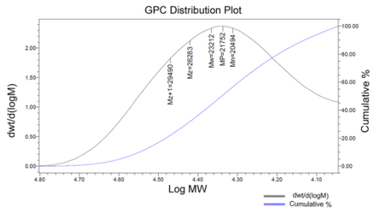

Figure 6. GPC result for PHPLA-cPt polymer. Average molecular weight of the polymer was found 22 kDa.

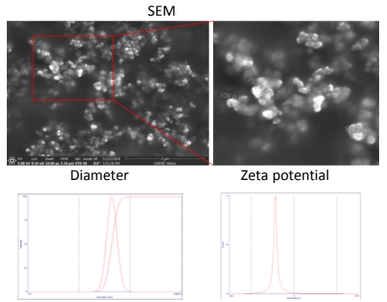

Figure 7. Size and morphology of CTX-PTX@PHPLA-cPt NPs. SEM images of the CTX-NPs; Mean diameter and zeta potential of the CTX-NPs by DLS.

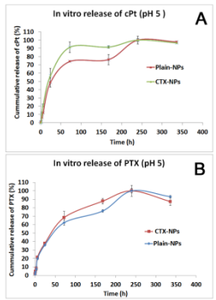

Figure 8. *In vitro* release study. A) Cumulative release profile of cisplatin (cPt) from both plain NPs and CTX-attached NPs at pH 5.0; B) Cumulative release profile of paclitaxel (PTX) from both plain NPs and CTX-attached NPs at pH 5.0.

References

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Results, continued

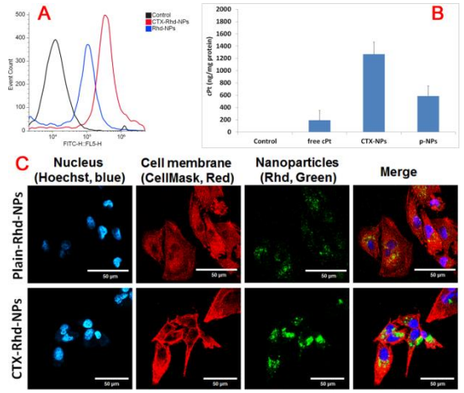

Figure 9. A. After 3 h of incubation at 37 ºC, the cells were analyzed by flow cytometer to characterize the uptake of Rhd-NPs and CTX-Rhd-NPs. Here, rhodamine (Rhd) was applied as a payload that has a fluorescence signal instead of a PTX molecule. **B.** After 3 h of incubation at 37 ºC, cellular uptake of cisplatin was measured by AAS. **C.** Cellular uptake of plain-Rhd-NPs and CTX-Rhd-NPs by confocal microscope after 48 h of incubation in MDA-MB-231 cells. Hoechst (blue) indicates nucleus, CellMask Red (red) indicates cell membrane, and Rhd (green) presents rhodaminde 123 as a payload.

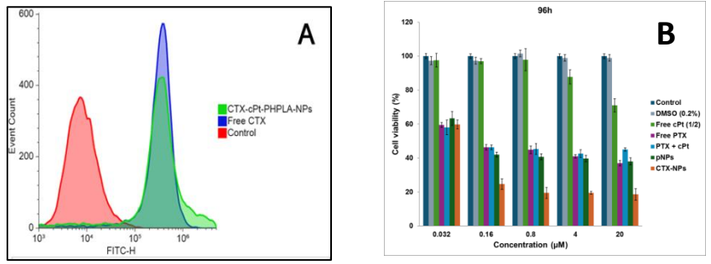

Figure 10. (A) Cell binding (A) of CTX-NPs on MDA-MB-231 cell line by FACS. Cell cytotoxicity results at 96 h (B) after treatment with free cPt and PTX, combination of cPt and PTX, drug-loaded p-NPs, and CTX-NPs against MDA-MB-231 cell line at 37 ºC were shown. The IC50 values of CTX-NPs, p-NPs, and PTX + cPt after 96 h of incubation were 0.1 μM, 0.49 μM, and 0.57 μM, respectively.

Conclusions

Our study led to the development of polymeric multifunctional nanoparticles dual-loaded with surface-tagged monoclonal antibody for targeted treatment of TNBC. We chose two anticancer drugs (cPt and PTX) and loaded them into polymeric NPs using covalent linkage and hydrophobic interaction. To actively deliver the NPs to the TNBC cells, CTX (mAb), a ligand for EGFR, was attached to the surface of the NPs by thioether linkage. We obtained CTX-NPs that contain both cPt and PTX, with a diameter of around 190 nm. Furthermore, it was demonstrated that the CTX-NPs exhibit slow drug release for up to 10 days at low pH and efficient uptake by TNBC cells owning to EFGR receptor-mediated internalization. Cytotoxicity study showed that CTX-NPs have stronger efficacy against TNBC cells (MDA-MB-231), compared to plain NPs and free anticancer drugs. Our study confirmed that CTX-NPs possess excellent *in vitro* properties [3]. *In vivo* studies using the TNBC tumor model are under progress now.

Acknowledgement

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