Cholecalciferol Nanomicelles for the Treatment of Triple Negative Breast Cancer
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Purpose
Triple negative breast cancer is highly aggressive and metastatic type of cancer lacking expression for estrogen, progesterone and human epidermal growth factor receptor (HER2). Very few treatment options are available for TNBC due to its absence of molecular targets and development of multi drug resistance (MDR). Despite recent progress in the identification of specific molecular targets, there is no targeted treatment available for TNBC patients. TNBCs are initially sensitive to standard chemotherapy, but have a high rate of local recurrence and systemic metastasis that are unresponsive to current therapies. Our laboratory is extensively working on the cholecalciferol derivatized formulations in order to address treatment and resistance issues associated with TNBC. Cholecalciferol (Vitamin D) is very well-known for its role in calcium and phosphorus metabolism and has also been explored for its inhibition of cell proliferation and induction of cell differentiation. Vitamin D receptors (VDR) are reportedly present in about 80 percent of breast tumors and hence considered to be a potential target for effective breast cancer therapy. The purpose of the present study is to synthesize, design and characterize polyethylene glycol cholecalciferol nanomicelles to overcome doxorubicin (DOX) resistance in TNBC.

Methods
Polyethylene glycol (PEG)-cholecalciferol was successfully synthesized using biodegradable glutaric ester linker and characterized by 1H NMR, IR, and DSC. DOX was passively loaded into the micelles, evaluated for particle size, zeta potential, drug release, cellular uptake, cytotoxicity assay, migration assay, apoptosis and analyzed for its biomarkers specific activity in TNBC cells.

Results
NMR spectra of PEG-cholecalciferol had characteristic peak of PEG at $\delta 3.75$ ppm and the characteristic peaks of cholecalciferol were observed from $\delta 1 - \delta 4.2$ ppm and $\delta 5 - \delta 8.4$ ppm. FT-IR spectra had characteristic stretching vibration of ester bond at 1712cm$^{-1}$. A two-fold reduction in the IC50 value (p ≤ 0.05) and significant enhancement in anti-migration effect of DOX in cholecalciferol treated MDA-MB-231/DOX cell line was observed. Intracellular DOX concentration and cytotoxicity were significantly enhanced by PEG-cholecalciferol nanomicelles compared to DOX solution in DOX resistant cell line. We also observed that micelles enhanced the apoptotic activity of DOX and downregulated the expression of tumor markers c-Myc and m-Tor along with upregulation of marker Bax (p ≤ 0.05).

Conclusion
The results from the present study suggest the role of PEG-cholecalciferol conjugate as a promising nanocarrier for the delivery of various hydrophobic anticancer drugs and overcoming resistance in TNBC.

![Cytotoxicity studies of CPG micelles.](image-url)

Fig. 1. IC50 value of PEG-cholecalciferol-DOX formulations on respective cell lines.