Proceedings of the 7th World Congress on New Technologies (NewTech'21)
~~Prague, Czech Republic~~ Virtual Conference – August, 2021
Paper No. ICNFA 301
DOI: 10.11159/icnfa21.301

Gold Nanoparticle as a Model Nanoparticle System for Efficient Delivery of Anticancer Drugs

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Abstract **-** Only a small fraction of anticancer drugs gets (<0.01%) into the tumor when they are administered as free drugs to cancer patients. This results in many side effects to patients since drug molecules get into healthy, normal cells as well as tumour cells. We propose using gold nanoparticles (GNPs) for controlled and optimized delivery of drugs to overcome the side effects of poor distribution of anticancer drugs. Our studies show that normal cells take much less GNPs in contrast to tumor cells making them a more selective delivery vehicle for anticancer drugs. In this study, we have shown that GNPs offer the possibility of transporting major quantities of drugs due to their large surface-to-volume ratio. We have functionalized GNPs with natural peptides and polyethylene glycol for effective intracellular targeting and biocompatibility, respectively. In this in vitro study, we chose to use bleomycin (BLM) as the anticancer drug due to its limited therapeutic efficiency (harmful side effects). BLM was conjugated onto GNPs through a thiol bond. The effectiveness of BLM was observed by visualizing DNA double strand breaks and by calculating the survival fraction. The action of the drug (where the drug takes effect) is known to be in the nucleus, and our experiments have shown that some of the GNPs carrying BLM were present in the nucleus. The use of GNPs to deliver anticancer drugs increased the delivery and therapeutic efficacy compared to the free drug. Combined use of radiation therapy and chemotherapy is being used to treat locally advanced tumors. It is shown that GNPs can also be used as radiation dose enhancers. Therefore, this GNP-based drug carrier will make a paradigm change in achieving a significantly higher therapeutic ratio while minimizing side effects of both chemotherapy and radiotherapy while improving the quality of life of cancer patients.

Keywords**:** Gold Nanoparticles, Bleomycin, DNA damage, Tumor cells

1. Introduction

One of the main issues in current chemotherapy is the side effects due to the anti-cancer drugs[1]. Controlled delivery of drugs to the tumor would reduce the required dose of chemotherapeutic agents and consequently, the normal tissue toxicity [2-5]. Bleomycin (BLM) is one of the most potent natural anti-tumor drugs used in clinical treatments of cancers like Hodgkin's disease, non-Hodgkin's lymphoma, and testicular cancer[6, 7]. However, BLM's pulmonary toxicity limits its therapeutic effectiveness [2]. The usage of this anti-tumor drug could be widened if the delivery can be controlled. One of the ways to achieve controlled delivery of drugs is to use nanoparticles (NPs) as delivery vehicles. Studies have shown that NP-based drug delivery systems can provide improvements to the free drug [8-16].

Among other NP systems, we used gold NPs (GNPs) as our drug delivery system since their chemical and physical properties allow easy functionalization with anticancer drugs. GNPs are also biocompatible and have shown their biocompatibility in a phase I clinical trial. These NPs get accumulated within tumor tissues through passive targeting because of enhanced permeation and retention (EPR) effects[16]. In addition, we have chosen smaller size GNPs to take advantage of surface-to-volume ratio for loading anticancer drugs and targeting molecules. In this study, we have chosen bleomycin (BLM) as the anticancer drug. A peptide containing integrin binding domain "RGD" was added to the NP surface for

targeting integrin receptors, which are overexpressed in most tumor cells[11]. To achieve biocompatibility and stabilize RGD conjugation, polyethylene glycol (PEG) molecules were also added to the NP surface. The functionalization of BLM, RGD peptide, and PEG was easily achieved through thiol linkages[17]. Our final objective was to deliver BLM efficiently using GNP as a carrier [16]. A previous study has shown that breast cancer cells (MDA-MB-231) cells treated with 12 μM 1.9 nm gold nanoparticles (GNPs) produced a sensitizer enhancement ratio (SER) of 1.38 compared to the samples treated with BLM only[18]. In our study, we used ~11 nm GNPs and were able to reduce the concentration of the drug by thousand-fold but still were able to see the improved therapeutics. For simplicity, GNP-PEG-RGD complex is refer to as GNP and GNP-PEG-RGD-BLM is referred to as GNP-BLM in the rest of the proceeding.

2. Results and discussion

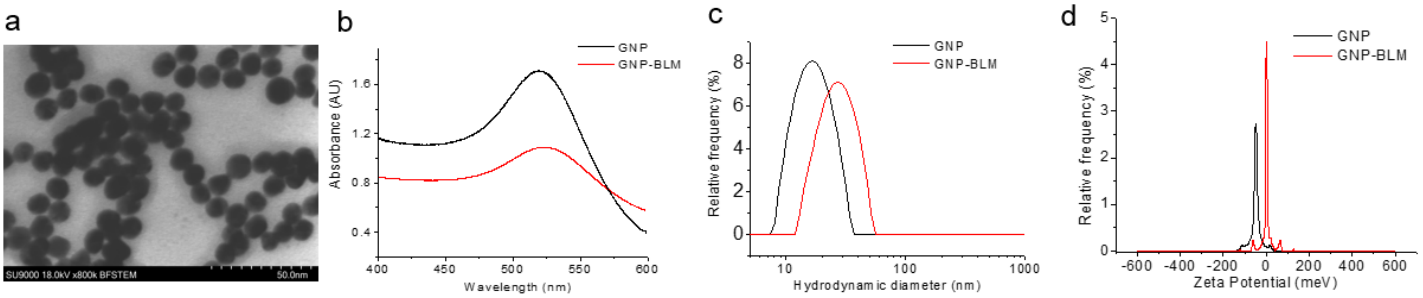

Fig. 1: Characterization of GNP-BLM complexes. a) Transmission Electron Microscopy image of GNPs used for the study. b-d) UV Visible spectroscopy, dynamic light scattering data, and zeta potential data for GNP and GNP-BLM complex, respectively.

GNP complexes used for the study were characterized using transmission electron microscopy (TEM), ultraviolet (UV) absorption spectrometry, dynamic light scattering (DLS), and zeta potential measurements.

TEM images of GNPs are shown in Fig. 1a and the core diameter is ~11 nm. The UV-Vis spectrum of citrate capped GNPs had a peak of 517 nm (Fig. 1b) which corresponds to approximately ~11 nm in core diameter. GNP-BLM complex was assembled through sequential conjugation of PEG, RGD, and BLM at optimized ratios of 250:150:4000 per GNP. The peak red shifted to 519 nm after conjugation with RGD peptide, PEG, and BLM. The complex was stable since the shape of the spectrum remains the same up to 48 hours post conjugation. The hydrodynamic diameter was also measured (see Fig. 1c). Adding bleomycin, increased the diameter by 1 to 2 nm. This corresponds to the approximate size of conjugated molecules. For example, size of RGD peptide, PEG, and BLM are 1760, 2000, and 1512 Da. We also measured the changes in zeta potential (Fig. 1d) which are consistent with added molecules onto GNP surface.

Before moving to uptake studies, we analysed the cell growth in the presence of free BLM vs GNP-BLM to identify the effectiveness of using GNPs as the carrier for BLM. The IC-50 of free BLM is ~ 11μm for PC-3 cell line that we studied [19]. However, IC-50 was reduced by a thousand-fold by conjugating BLM onto GNP surface as illustrated in Fig. 2a. We used 1 nM concentration of GNP-BLM complex for our cell experiments. In our study, we used live-cell imaging to assess nuclear damage in cells treated with GNP-BLM compared to cells dosed with the equivalent concentration of free BLM. We also perform live cell imaging to evaluate the damage due to BLM. As illustrated in Fig. 2b, there was a significant damage to nuclei of cells treated with GNP-BLM complex vs GNP alone or free BLM. The mechanism of cell damage due to BLM is through DNA damage within the cell nucleus [20]. We noticed that a free BLM concentration in the nM range did not damage cells as shown in Fig. 2b, while more micronucleation was observed when cells were treated with GNP-BLM complex. These observations suggest that BLM conjugated to GNPs caused more nuclear damage than the equivalent concentration of free BLM, which is consistent with our proliferation assay results in Fig. 2a.

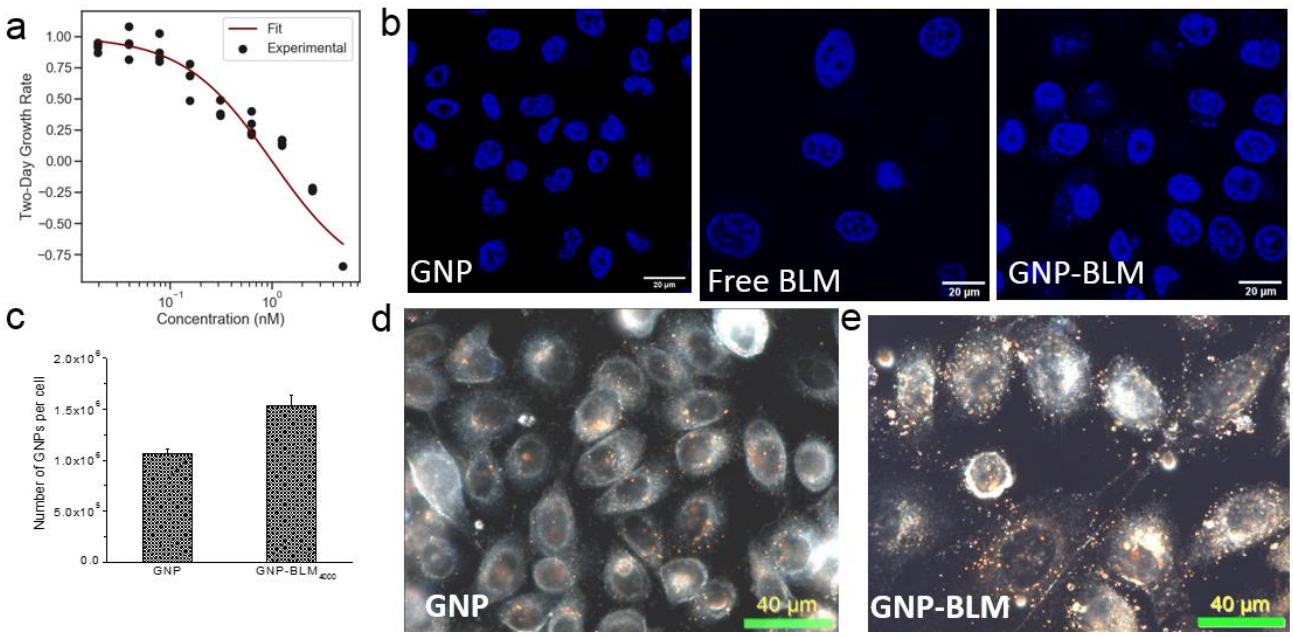

Fig. 1: Use of GNP as a carrier for BLM. a) Growth curve of cells treated with different concentrations of GNP-BLM. b) Nuclei of cells treated with GNP (left), free BLM (middle), and GMP-BLM (right). c-d) Quantification data for GNP and GNP-BLM complex, respectively. Bright yellow spots represent GNP clusters in cells.

We used inductively-coupled mass spectroscopy (ICP-MS) as a quantification technique to determine the extent of GNPs present in the cells. Based on quantification data in Fig.2c, there was a slight increase in GNP accumulation of GNPs in cells. This could be due to the slightly positive charge of GNP-BLM vs GNP as explained in Fig. 1d.

4. Conclusion

Our study led to designing a NP-based platform to deliver the anticancer drug, bleomycin, while maintaining its cytotoxic activity. Furthermore, both GNPs and BLM are considered as radiosensitizing agents in radiotherapy. Radiotherapy and chemotherapy are being used as major treatment modalities to treat cancer patients. Feasibility of transporting GNPs into cancer cells by functionalizing them with anticancer drugs will shed light on combined use of radiation therapy and chemotherapy in treating most resistant cancer cells.

Acknowledgements

The authors would like to acknowledge the support from University of British Columbia, BC Cancer (Vancouver &Victoria). This study was funded by a Nanomedicines Innovation Network Strategic Initiative fund (NMIN-SI) from government of Canada, the John R. Evans Leaders Fund (JELF) from the Canada Foundation for Innovation (CFI) and British Columbia Knowledge Development Fund (BCKDF), the NSERC Discovery grant from the Natural Sciences and Engineering Research Council of Canada (NSERC), and a collaborative health grant from the University of Victoria.

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