Targeted Photodynamic Diagnosis of Colorectal Cancer

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ABSTRACT

Background: Colorectal cancer is one of the major causes of death worldwide. To supplement the limitations of existing diagnostic methods, new diagnosis strategies that are non-invasive and can precisely target CRC cancerous cells with minimal side effects are significant for early diagnosis. Photodynamic diagnosis (PDD) is a valuable diagnostic procedure that is established when a fluorescent tumor-targeting photosensitizer is irradiated with light at a determined specific wavelength which then leads to the emission of fluorescence and this fluorescence effect may be used to detect cancerous tissues. ***Objective:*** This study aimed to determine if the targeted PS nanoconjugate (ZnPcS4 – AuNP-PEG5000-SH-NH2 – Anti-GCC Ab) could endow specific actively targeting abilities and heighten the intracellular accumulation of PSs for photodiagnosis. ***Methods:*** This study was performed on Caco2 cell line in a monolayer culture and WS1 human fibroblast cells served as a control. Spectroscopic technique was assessed. The fluorescent effects of the formulation were investigated by immunofluorescent morphological staining and fluorescent microscopy imaging assays with the 358Ex / 461Em DAPI filter. ***Results:*** fluorescent microscopy imaging results showed that the targeted PS nanoconjugate specifically bound with targeted overexpressed Caco2 cancerous cells only and had no affinity for normal human cells. ***Conclusion:*** This study shows that the final targeted PS nanoconjugate offered highly specific and sensitive absorption of the PS in CRC cells and so allowed for the successful photodynamic diagnosis of CRCs *in vitro*.

INTRODUCTION

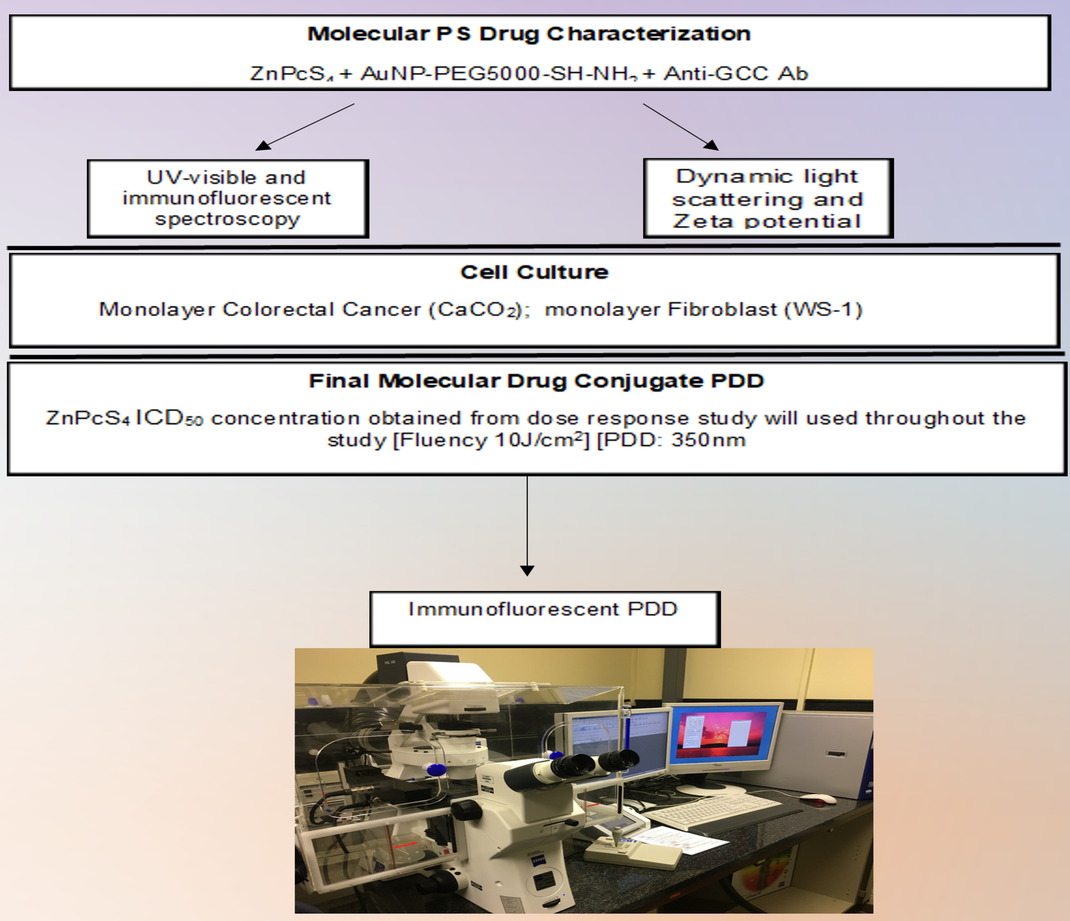
Colorectal cancer (CRC) is the third prevalent cause of mortality and fourth most commonly diagnosed form of cancer worldwide[1]. In South Africa CRC is ranked as the fourth most common cancer and the sixth most deadly[2].The traditional diagnostic methods and treatment approaches often do not provide the expected outcome and have several limitations with unwanted side effects[3]. In this regard, photodynamic techniques such as photodynamic therapy (PDT) that utilize a photosensitizer (PS) and specific light, can offer possibilities to mitigate CRC death with advantages of tumour selective targeting, minimal side effects, and provision of therapeutic destruction to targeted cancerous cells for an effective treatment[3]. Similarly, precise early diagnosis of CRC cancer is possible when photodynamic diagnosis PDD, which is based on the use of fluorescent PSs that selectively accumulate within cancerous cells and upon excitation by shorter purple to blue wavelength of light, result in fluoresce effects that can be used to detect cancerous cells, without damage[3].

Despite undeniable advantages, traditional PSs have inherent drawbacks such as poor water solubility, aggregation tendency and poor selectivity for targeted cancerous cells, which can limit PDD and PDT applications[4]. Nanoparticles (NPs) can provide innovative PS targeting and delivery nanocarriers for CRC diagnosis and treatment with merits of improved PS uptake and passive delivery[5]. Furthermore, to enhance PS accumulation specificity within targeted cells, as well as enhance PS active delivery and improve PDT PS safety and tolerability profile, strategies based on active targeting are currently under investigation[6].

Gold nanoparticles (AuNP) have been considered as idealistic delivery platforms for improved PS passive absorption in PDT NP-PS delivery strategies[7]. Owing to their high affinity of functional groups, AuNP also form part of an effort to facilitate active targeting, whereby conjugation of target ligands, such as antibodies and PS molecules onto their high surface areas may provide maximum uptake and selective delivery of PS to the desired target sites, with reduced toxicity effects to healthy tissue, as well as promote the efficiency of simultaneous cancer diagnosis and PDT treatment[3,7].

In our work, a novel actively targeted PS nanoconjugate ($ZnPcS_4$ – AuNP-PEG5000-SH-NH_2 – Anti-GCC Ab) based on a heterobifunctional amine-functionalized and PEG stabilized gold nanoparticles (AuNPs) was loaded with $ZnPcS_4$ (a tetra sulphonated PS), which was conjugated with specific CRC targeting surface antibodies (Anti-GC-C). This study aimed to determine if the targeted PS nanoconjugate ($ZnPcS_4$ – AuNP-PEG5000-SH-NH_2 – Anti-GCC Ab) could endow specific active targeting abilities and heighten the intracellular accumulation of PSs for improved PDD outcomes.

METHODOLOGY



RESULTS

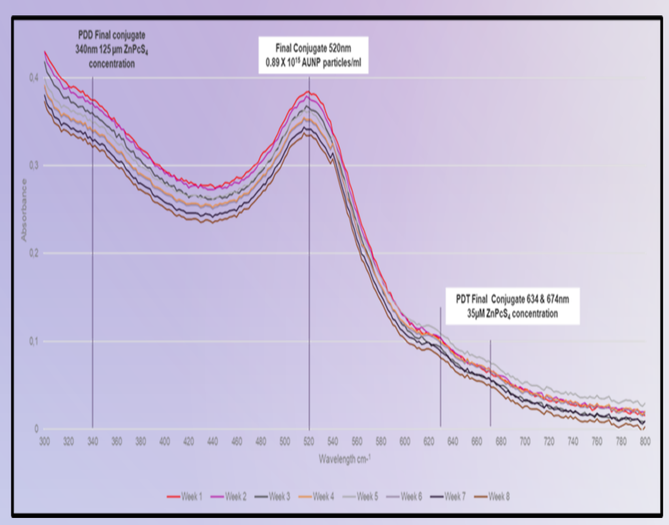


Figure 1. UV-Visible absorption photostability spectra of the targeted PS nanoconjugate (ZnPcS4 -AuNP-PEG5000-SH-NH2 - Anti-GCC Ab) was measured over 8-week period and recorded within the 300 to 800 nm spectral region . As shown in the UV−vis spectrum (Figure 1), the targeted PS nanoconjugate showed no significant shift changes within the high absorption (340 nm - Soret Band) and emission (583, 634 and 674 nm - Q band) peaks. The results demonstrated targeted PS nanoconjugate remained stable and free of aggregation, and the ZnPcS4 PS within the final PS nanoconjugate retained its photochemical properties.

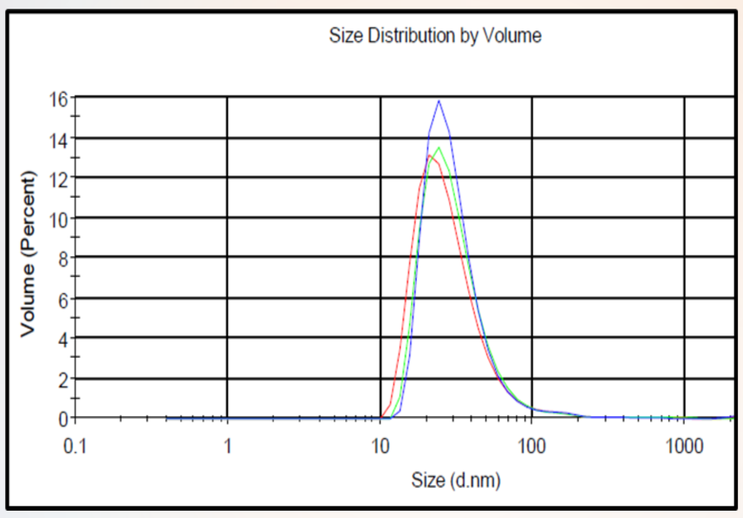


Figure 2. DLS technique was used to investigate the homogeneity and purity of the nanoconjugate in order to determine if the final nanoconjugate was homogenous with negligible aggregates, that could hinder the active targeting abilities of Abs. The DLS hydrodynamic radius distribution graph PS nanoconjugate formulation consisting of ZnPcS4 - AuNP-PEG5000-SH-NH2 - Anti-GCC Ab showed one single major peak with a narrow width and no additional smaller side peaks, demonstrating that it was homogenously pure, remained spherical in shape and reported no aggregation.

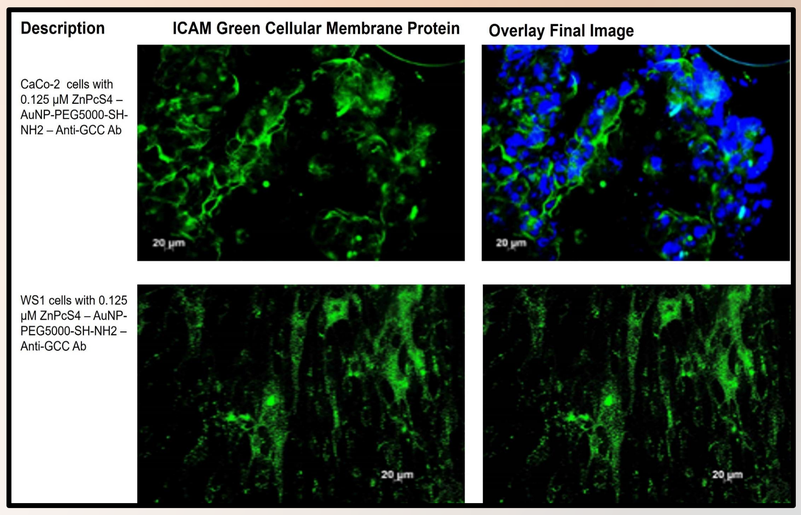


Figure 3. PDD assays comparison of ZnPcS4 PS uptake in CaCo-2 cells, treated with the targeted PS nanoconjugate and ZnPcS4 PS uptake in WS1 cells, treated with the final targeted PS nanoconjugate. ZnPcS4 fluorescence at 340 nm (Blue) and cellular membrane proteins (Green). The results showed that ZnPcS4 PS conjugated to a AuNP carrier, that is linked with an active targeting biomolecule (Anti-GCC Ab), active and specific uptake of PS in CRC cells is attained, with blue emission outlining the tumour. In contrast, negligible uptake of the PS was observed with WS1 cells(normal cells).

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CONCLUSION

This study found that the final PS nanoconjugate, had no actively specific affinity for healthy cells (such as fibroblasts), indicating that if was to be utilized within actively targeted CRC PDD detection, the application would remain favorable to targeted CRC only, and so be able to precisely and selectively be able to distinguish between normal and cancerous cells. The simultaneous PDD and PDT-mediated treatment of *in vitro* cultured CRC cells may be possible when utilizing the final actively targeted PS nanoconjugate, further work on PDT may yield improved PDT treatment outcomes.

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