aggregation and the slight shift in resonance peak position of AuNP after conjugation indicated the Ab and PS had been successfully conjugated to its surface.

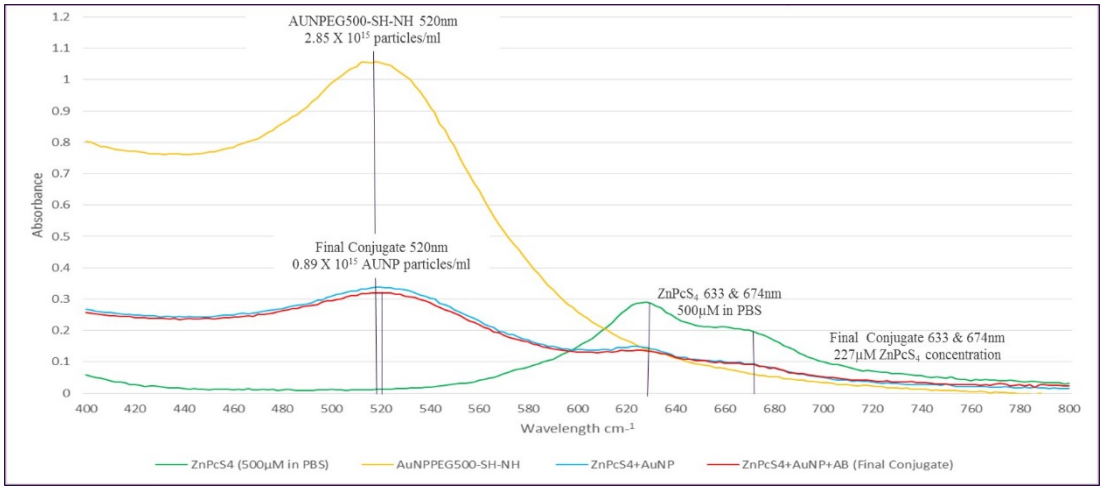

Figure 1: UV-visible spectrophotometry absorption and fluorescence spectra of final molecular conjugate and controls.

3.2. Sub Cellular Localization of Multicomponent Nanoparticle Drug Based System

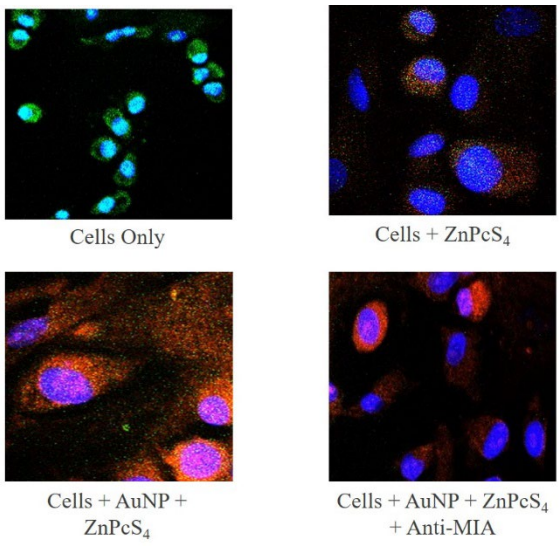

Figure 2: Immunofluorescent Staining of final molecular conjugate and controls.

Live cell imaging immunofluorescent staining was used to determine subcellular localization and uptake of the PS ($ZnPcS_4$) alone versus the final molecular conjugate (AUNPPEG500-SH-NH + $ZnPcS_4$ + Anti-MIA Ab) within in vitro cultured MM cells (Figure 2). MM cells showed poor uptake of $ZnPcS_4$ PS drug alone, however when conjugated to the AuNP and Anti-MIA Ab, improved and concentrated PS drug uptake was observed in cellular cytoplasm and nuclei. These findings suggest that the targeting affinity of the PS drug conjugate in relation to Anti-MIA Ab biomarker specify for MM A375 cells was functional, and so improved the subcellular localization and concentration uptake within these cells.

3.3. Cytotoxicity Biochemical Assays

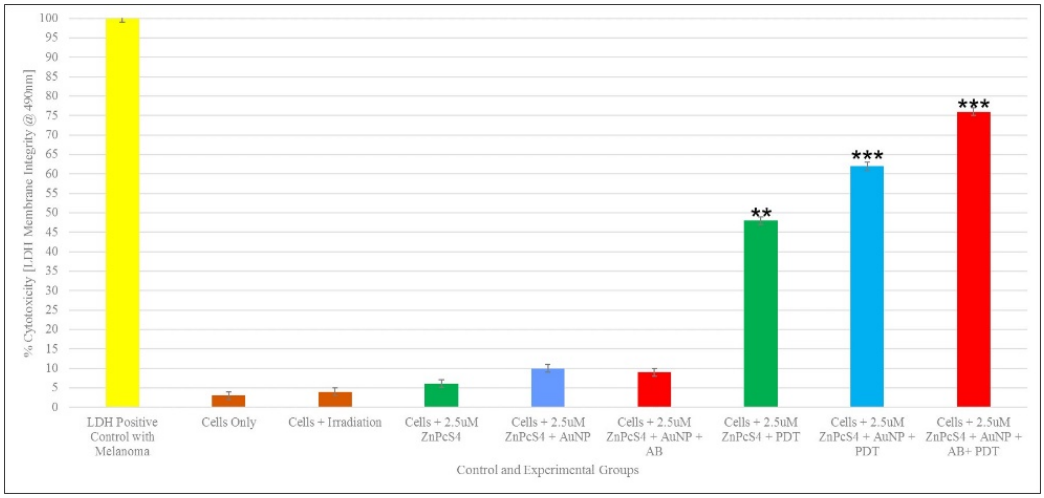

Figure 3: Cellular Cytotoxicity of Final Molecular Conjugate before and after PDT

With reference to Figure 3, the LDH positive control represents 100 % cellular cytotoxicity in MM cells, due to cell lysis and so was used as a standard reference to calculate percentage cytotoxicity in all control and experimental groups. The control group of cells only, was used as a standard reference for viable MM cells, which obviously reflect no cellular cytotoxicity, for statistical comparison in all other control and experimental groups. In control groups, which received laser irradiation only, no significant increase in cellular cytotoxicity was noted. This indicates that laser irradiation at 673 nm with a fluency of 10 J/cm^2 has no phototoxic side effect on cultured MM cells. Within control groups which received 2.5 µM of $ZnPcS_4$ PS drug only, no significant cytotoxicity was noted. These results suggest that the administration of PS drug to MM cells in the absence of light has no dark toxic side effects. Additionally, there was no significant cellular cytotoxicity observed in MM control groups, which received control 2.5 µM $ZnPcS_4$ – AuNP-PEG5000-SH-NH_2 or experimental 2.5 µM $ZnPcS_4$ – AuNP-PEG5000-SH-NH_2 – Anti-MIA Ab final molecular PS drug conjugate administration alone. These findings indicate that even when the $ZnPcS_4$ PS drug was bound to either AuNP-PEG5000-SH-NH_2, as well as Anti-MIA Ab, it remained stable pre-PDT and had no cytotoxic effects on cells. The results from these control groups were used as a reference so that the findings from the final molecular conjugate PDT experimental groups could be compared to these control references, making it possible to identify significant increases in cellular cytotoxicity ($P < 0.05$*) ($P < 0.01$**) ($P < 0.001$***).

All PDT treated experimental groups showed a highly significant cellular cytotoxicity values when compared to the control of MM cells only, however these significant increases percentage values varied over the different groups. Within PDT experimental groups which received 2.5 µM $ZnPcS_4$ PS drug only and 10 J/cm^2 laser irradiation, a significant increase of 45 % (***) cellular cytotoxicity was noted. Whereas, within PDT experimental groups which received 2.5 µM $ZnPcS_4$ – AuNP-PEG5000-SH-NH_2 and 10 J/cm^2 laser irradiation an even higher significant increase of 59 % (***) was found. These findings suggest that the binding of $ZnPcS_4$ PS drug to AuNP-PEG5000-SH-NH_2 promoted its passive uptake in cultured MM cells and so when subjected to laser irradiation their PDT induced cytotoxicity significantly increased. However, the most significant increase of 76 % (***) in cellular cytotoxicity was found in PDT experimental groups, which received 2.5 µM $ZnPcS_4$ – AuNP-PEG5000-SH-NH_2 – Anti-MIA Ab. Overall, the final molecular $ZnPcS_4$ PS drug conjugate induced far more significant cytotoxicity (76%) in MM cells after PDT treatment, when compared to PS drug alone PDT treatment

(48%) at the same PS concentration. These findings suggest that the conjugation of Anti-MIA Ab to $ZnPcS_4$ – AuNP-PEG5000-SH-NH_2, within the final molecular conjugate actively, as well as specifically enhanced PS drug uptake in MM cancer cells and so improved PDT cytotoxic treatment outcomes enormously.

4. Conclusion

$ZnPcS_4$ PS was successfully conjugated to PEG500-SH-NH, to maximize its solubility and stability, as well as bound to active tumour-associated antibody-antigens (anti-MIA Ab) to aid in specific targeted PS delivery. Within in vitro cultured MM (A375) cells, this final molecular drug conjugate proved to have far more enhanced and concentrated cellular uptake of $ZnPcS_4$ PS, than when just administered without drug delivery modification. Moreover, the final molecular $ZnPcS_4$ PS drug conjugate induced far more significant cytotoxicity (76%) in MM cells after PDT treatment when compared to PS drug alone PDT treatment (48%) and the same PS concentration was applied.

This suggests that the multicomponent AuNP PS drug-based system did have specific MIA antigenic targeting abilities for the active and far more concentrated photosynthetic drug delivery of $ZnPcS_4$ in MM cancer cells. Thus, the final molecular conjugate did in fact enhance PDT treatment in MM cells, though increased theranostics, as well as far more specific and targeted PS drug delivery, improving cellular uptake and overall cytotoxicity cell death induction. In relation to this studies increased specificity of PDT treatment, future investigative approaches will include the use of another normal cell line in order to be able to compare efficiency result outcomes.

5. Acknowledgements

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