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Exploring nanosystems for biomedical applications focusing on photodynamic therapy and drug delivery

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ABSTRACT

The increasing incidences of cancer and related deaths call for the development of new and improved treatment modalities. Photodynamic therapy (PDT) today is an alternative to conventional treatments, but has limitations. This thesis explores different nanosystems with aim to improve PDT focusing on spectroscopic and *ex vivo* studies.

Nanosystems capable of efficient photodynamic action in anaerobic or hypoxic conditions are gaining much attention. Constructs of cyclodextrin polymer encapsulating anthracene-nitroaniline conjugates, that can release nitric oxide (NO) radicals upon irradiation, were investigated in this thesis. It was demonstrated that concomitant increase of fluorescence can be used for dosimetry of NO release. Pulsed near-infrared laser light can be used for NO photorelease by two-photon excitation process that along with high phototoxicity (observed cell mortality >90%) make this nanosystem a promising technique in PDT (paper I).

A multimodal nanosystem consisting of a cyclodextrin polymer, adamantyl-nitroaniline, and zinc phthalocyanine tetrasulfonate was evaluated (paper II). Multiphoton microscopy showed cytosolic distribution of the nanosystem in *in vitro* cells and the ability of the nanosystem to penetrate into *ex vivo* skin. In addition, the combinatorial phototoxic effect elicited by singlet oxygen and NO (cell mortality >90%), indicates high potential of this multimodal nanosystem in PDT.

Herein, it is demonstrated that conjugation of water non-soluble photosensitizer (*m*THPP) to cyclodextrin can enhance its aqueous solubility and monomerization, thereby leading to improved photophysical properties in aqueous environment (paper III).It was also shown that conjugation facilitates skin penetration *ex vivo*. Fluorescence lifetime imaging demonstrated accumulation of the monomeric conjugate in the cytoplasm *in vitro* cells.

It has been suggested that PDT enhancement can be achieved by a combination of photosensitizer and gold nanoparticles; however, the investigations in this thesis demonstrate a lack of the effect using protoporphyrin IX and PEGylated goldnanorods (paper IV). Cell viability studies were combined with spectroscopic measurements confirming a lack of energy transfer between nanoparticles and photosensitizer. Incubation of cells combining aminolevulinic acid and gold nanorods showed a slightly elevated PDT efficiency, however this effect is most likely attributed to an enhanced delivery of aminolevulinic acid rather than the energy transfer.

Finally, a nanosystem consisting of gold nanoparticle labelled with lactose moieties was explored for tumour-specific delivery (Paper V). Multiphoton microscopy was used to visualise the multiphoton-induced luminescence from the particles loaded to epithelial cancer cells and keratinocytes. The study demonstrates that tumour-specific uptake can be obtained by targeting galecin-3, known to be overexpressed in tumour cells.

Taken together, the work in this thesis presents several promising nanosystems to improve PDT. Of particular interest are the NO photoreleasing nanosystems for hypoxic conditions. Furthermore, improved biodistribution and targeted delivery can be obtained by clever design of the systems, presenting interesting approaches to aid in restraining the acute problem of increasing worldwide occurrence of cancer.

Keywords: nitric oxide, photodynamic therapy, NO-based PDT, PDT enhancement, *m*THPP, cyclodextrin, CD-*m*THPP conjugate, PpIX AuNP combination, targeted drug delivery, galectin-3, two-photon microscopy, FLIM, cell phototoxicity, *ex vivo* skin.