Nano-Biophotonics

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I certify that all material in this thesis which is not my own work has been identified and that no material has previously been submitted and approved for the award of a degree by this or any other University.

..... **Natalie Garrett**

Abstract

Photonic techniques are the methods of choice for probing biological systems, as they are non-invasive, non-ionising, inexpensive, and are ubiquitous. When applied to the treatment and prevention of disease and for pathology in general, biophotonics offers a means to bridge the gap between understanding of molecular structures and their role in physiological functions. There is a wide range of such techniques used in imaging, assaying, bio-sensing, optical diagnosis, each of which has limitations as well as benefits. The experiments outlined in this thesis use nanotechnology to overcome the limitations of resolution, contrast and chemical specificity with photonic techniques in biology.

The experimental work outlined in this thesis is divided over three chapters, the first of which is concerned with nanostructured metallic surfaces for use in surface enhanced Raman scattering (SERS) for protein assay applications. This chapter gives details of the methods used to produce and characterise SERS substrates using gold and silver thermally evaporated onto butterfly wing sections, together with the protocols developed for manufacturing biomimetic analogues of these naturally occurring nanostructures. The conjugation system designed to modify the metal surfaces for use in an avidin/biotin model protein binding assay is described, together with an account of the efficacy of the final assay. The results obtained show that such naturally occurring nanostructures, and their biomimetic analogues, are suitable for use as SERS substrates for wet protein binding assays. This work represents a major advance in the field of SERS assay.

The next experimental chapters describe experiments that use coherent Raman scattering (CRS) methods to probe the interactions between nanoparticles and live cell cultures, as well as provide chemically selective images of tissue samples. Chapter 6 gives an account of work undertaken to investigate gold nanoshells, novel nanoparticles comprising spherical silica cores surrounded by a layer of gold, whose plasmon resonance wavelength may be tuned by altering the shell thickness and core diameters. Gold nanoshells exhibit unique optical properties arising from this plasmon resonance, and their ability to rapidly heat under exposure to light at this resonant wavelength has been used for photodynamic therapy of tumours in mice, in one instance with white

blood used cells to carry gold nanoshells to the site of the tumour. Although previous photothermal experiments involving tumour destruction using nanoshells have given promising results, they largely ignored the interactions between single cells and the gold nanoshells. The experiments in this chapter probe these interactions, using coherent Raman scattering (CRS) microscopy.

CRS is a chemically-selective multiphoton modality that can be used to image live cells noninvasively and with excellent chemical specificity and contrast. To determine the extent to which CRS microscopy-induced heating of gold nanoshells would affect live cells, macrophage cells were induced to phagocytise gold nanoshells prior to exposure to CRS. A trypan blue vital staining technique was used to determine cell survival rates after exposure to a range of laser powers. The photoluminescent qualities of gold nanoshells were also exploited as a marker for phagocytosis, the rate of which was investigated using CRS, as a function of the concentration of two different H_2S donors in the cell medium.

The final experimental chapter investigates the ability of CRS to image organic nanoparticles within cells and tissues. Two varieties of nanoparticles and cell lines were used in this chapter: dendrimer/plasmid DNA nanoparticles with human skin cancer cells and apo lipoprotein E (APOE) conjugated to albumin nanoparticles with human brain endothelial cells. Dendrimer nanoparticles have been shown to exhibit characteristics that make them efficient potential candidates for gene therapy, since they are able to spontaneously bind to DNA and deliver it across cell membranes. Before this gene delivery vector is used in humans it is important to investigate the distribution of these nanoparticles as they pass inside cells, using a non-invasive imaging technique that provides sufficient spatial resolution and chemical selectivity. APOE-conjugated albumin nanoparticles have been found to cross the blood-brain barrier, and hence their uptake by brain endothelial cells is of great relevance for engineering drugs to treat brain disease.

The potential of SRS microscopy to image the blood vessels within the brain was investigated using slices of mouse brain. This provided clear images of the blood-brain barrier, a selectively permeable membrane comprising endothelial cells lining the blood vessels innervating the brain. The BBB effectively blocks access to over 95% of drugs

from entering the brain, targeted engineering of drugs to cross this barrier is necessary in order to treat diseases of the brain. Therefore, the images provided in these brain imaging experiments have demonstrated the potential for coherent Raman scattering microscopy in quantifying drug nanoparticle uptake within dosed animal brains.

The interaction between ApoE-albumin nanoparticles and brain endothelial cells was investigated in using coherent Raman scattering. Signal intensity from three stimulated Raman scattering microscopy images of endothelial cells exposed to nanoparticles was colocalised using the relative intensity of signal probing three Raman bands identified in spontaneous Raman scattering spectra of the nanoparticles. The pump wavelengths used were 813 nm, 821 nm and 938.9 nm and the Stokes wavelength was 1064 nm in each case. The data obtained showed that ApoE-albumin nanoparticle signal associated with the cells was overlapped by signal from CH_2 within the sample, indicating the involvement of lipid-rich structures within the cells in receptor membrane endocytosis.

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List of Abbreviations

CARS – coherent anti-Stokes Raman scattering

CRS – coherent Raman scattering

DMEM – Dulbecco's modified Eagle's medium

DMSO – dimethyl sulphoxide

DPBS – Dulbecco's phosphate buffered saline

EDC - ethyl dimethylaminopropyl carbodiimide

EDTA - ethylenediaminetetraacetic acid

ELISA - enzyme-linked immunosorbent assays

EM – electromagnetic

FBS – foetal bovine serum

FWM – four wave mixing

GNS – gold nanoshells

LSP – localised surface plasmon

MPA – 3-mercaptopropanoic acid

NHS – N-hydroxy succinimide

NIR – near infrared

OPO – optical parametric oscillator

PBS – phosphate buffered saline

PMMA - poly(methyl methacrylate)

SAM – self assembled monolayer

SEM – scanning electron microscope

SERDS – shifted excitation Raman difference spectroscopy

SERS – surface enhanced Raman spectroscopy

SPP – surface plasmon polariton

SRG – stimulated Raman gain

SRL – stimulated Raman loss

SRS – stimulated Raman scattering

TEM – transmission electron microscope

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