Microbead-based Raman/Surface Enhanced Raman Scattering Immunoassays for Multiplex Detection

A THESIS SUBMITTED

BY

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I dedicate this whole thesis to my beloved husband,

for his support, encouragement and love.

DECLARATION

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ABSTRACT

The aim of this thesis project was to develop polymer microbead-based Raman/surface enhanced Raman scattering (SERS) immunoassay systems for the multiplex, specific and sensitive detection of biological molecules. Immunoglobulin G (IgG) was used as model proteins. In the system, gold nanoparticles (AuNPs) serve as SERS-active substrates. Different Raman-active molecules, such as 4-mercaptobenzoic acid (4MBA), can be easily self-assembled on the AuNPs as SERS tags. Polymer microbeads offer as immune-solid supports and provide Raman signatures. This study focused on the fabrication of different SERS tags, SERS-active microbeads and Raman spectroscopic-encoded microbeads for microbead-based Raman/SERS immunoassay development.

Polymer microbead-based Raman/SERS immunoassay system was first developed using 50 nm AuNPs and 130-600 μm carboxylated polystyrene (PS) microbeads synthesised by suspension polymerisation. Antibodies (FITC-labelled donkey anti-goat IgG) were conjugated to polymer microbeads by EDC/NHS coupling chemistry. The SERS tags were comprised of Raman-active molecules (4MBA) and AuNPs. Antigens (DyLight^{TM}649-labelled goat anti-human IgG) were successfully conjugated on SERS tags to form SERS reporters. The immunoassay was performed by mixing the protein conjugated polymer microbeads and SERS reporters together. Due to the specific recognition between antibody and antigen, AuNPs can be attached on the surface of polymer microbeads. The results were verified using fluorescence imaging and Raman/SERS analysis.

Since flow cytometry can rapidly sort large number of cells and particles in a short time, our intention was to take the advantages of both flow cytometry and Raman effects to develop Raman flow cytometry for multiplex and rapid detection. Therefore, monodisperse polymer microbeads with unique Raman signatures need to be synthesised. The

preparation of the monodisperse polymer microbeads with specific Raman signatures was carried out by two approaches. Firstly, the SERS-active microbeads were synthesised by the deposition of AuNPs on the surface of polymer microbeads and the addition of the Raman-active molecules prior to silica coating. The preparation of polystyrene microbead/AuNP composite microspheres was achieved through two methods (direct adsorption and in-situ growth). The mechanism for the silica coating of polystyrene/AuNP composite microspheres was discussed in details. 4-mercaptophenol (4MP) was self-assembled on the composite microspheres, followed by silica coating to obtain the SERS-active microbeads.

Secondly, the Raman spectroscopic-encoded copolymer microbeads were fabricated using styrene (Sty), 4-tertbutylstyrene (4tBS), and 4-methylstyrene (4MS) by dispersion polymerisation. Acrylic acid (AA) was used as the co-monomer to generate carboxyl groups on the surface of polymer microbeads. Six kinds of copolymer microbeads with the average diameters between 1.07 and 1.69 μm, including poly(Sty-AA), poly(Sty-4tBS-AA), poly(4tBS-AA), poly(Sty-4MS-AA), poly(4MS-AA), and poly(4tBS-4MS-AA), were synthesised with narrow size distribution and unique Raman fingerprints, which could be employed as spectroscopic-encoded microbeads in microbead-based Raman/SERS immunoassay system.

Monodisperse polystyrene microbeads with 1.6 μm diameter were also used to perform the polymer microbead-based Raman/SERS immunoassays. A similar immunoassay system as previous was applied for IgG recognition based on AuNPs and monodisperse PS microbeads, which were sorted and analysed using flow cytometry and Raman equipment.

In summary, the thesis proposed a new strategy for multiplex detection and reported the preliminary studies on polymer microbead-based Raman/SERS immunoassay. Different SERS-active microbeads and Raman spectroscopic-encoded copolymer microbeads have been successfully synthesised.

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ABBREVIATIONS

In this thesis, the following abbreviations are used.

AA	acrylic acid
Ab.	antibody
Ag.	antigen
AgNPs	silver nanoparticles
AIBN	2,2'-azobis(2-methylpropanitrile)
APTMS	3-aminopropyl-trimethoxysilan
4ATP	4-aminothiophenol
AuNPs	gold nanoparticles
BPO	benzoyl peroxide
BSA	bovine serum albumin
CM	chemical enhancement mechanism
CV	crystal violet
CV	coefficient of variation
DI water	deionised water
D_{n}	number-average diameter
EDC	1-ethyl-3-(3-dimethylaminopropyl) carbodiimide) hydrochloride
EEF	effective enhancement factor
EGDMA	ethylene glycol dimethacrylate
ELISA	enzyme-linked immunosorbent assay
EM	electromagnetic enhancement mechanism
FC	flow cytometry
$HAuCl_4 \cdot 3H_2O$	gold (III) chloride trihydrate
IR	Infrared
LOD	limit of detection
LSPR	localized surface plasmon resonance
4MBA	4-mercaptobenzoic acid
4MP	4-mercaptophenol
4MS	4-methylstyrene
Na_3Ct	trisodium citrate dehydrate

NHS	N-hydroxysuccinimide
PBS	phosphate buffer saline
PS	polystyrene
PVA	polyvinyl alcohol
PVP	polyvinyl pyrrolidone
QDs	quantum dots
R6G	Rhodamine 6G
RT	room temperature
SAMs	self-assembled monolayers
SEM	scanning electron microscope
SERS	surface enhanced Raman scattering
S/N	signal-to-noise
Sty	styrene
4tBS	4-tert-butylstyrene
TEM	transmission electron microscope
TEOS	tetraethyl orthosilicate
THF	tetrahydrofuran
Tris	tris (hydroxymethyl)aminomethane
UV-Vis	ultraviolet-visible