CHAPTER I

INTRODUCTION

1.1 Statement of problem

Separation and analysis of biomarkers such as peptides found in trace level play crucial roles in medical diagnosis. A number of peptides have been proposed as potential biomarkers for breast cancer such as bradykinin, des-Arg9-bradykinin and Hyp3-bradykinin [1] of which detection necessarily requires highly sensitive technique. Glutathione (Glu) and cysteine (Cys), thiol-containing peptides, are also recognized as important biomarkers in human [2, 3]. Glutathione (Glu) is an antioxidant, preventing damage to important cellular components caused by reactive oxygen species such as free radicals and peroxides. Cysteine (Cys) is present in almost all human proteins (89.3 % contain at least one), but only 17.2 % of tryptic peptides contain one or more cysteine residues. Analysis of biomarker requires highly sensitive analytical tools such as electrochemistry, fluorescence spectroscopy, UV-Vis spectrophotometry, surface plasmon resonance and mass spectrometry (MS) [4-7].

Surface-assisted laser desorption/ionization mass spectrometry (SALDI-MS) is a widely used technique for biomarker identification especially peptides due to its high sensitivity and accuracy [8]. The major benefit of this method lies in its ability to be implemented in specific modification of chip surfaces, recently developed by using nanomaterials as matrices to absorb the laser energy and transfer to analyte in ionization step. This technique is capable of detecting analyte with low mass range

(< 500 m/z) without the interference from matrix signal. Metal nanoparticles, for example, ZnS, TiO_2, Fe_3O_4 and Au are extensively used as matrices in SALDI-MS analysis [9, 10]. Unlike conventional organic matrices, metal nanoparticles provide low matrix background, homogeneous sample and insignificant fragmentation. In addition, some metal nanoparticles such as gold nanoparticles (AuNPs) and silver nanoparticles (AgNPs) can also act as concentrating and selective probes for pre-concentration and separation of thiol-containing peptide from the peptide mixture [11].

Recently, polymer has been used together with nanoparticles for modifying chip surface not only to improve nanoparticles dispersion that can enhance ionization efficiency, but also help trapping the nanoparticles from coming off during ionization. Polymer brushes are one of interesting material to be used for chip surface modification. Having chain ends attached covalently on surface, they are more stable than physically adsorbed polymer film. Polymer brushes with well-controlled molecular weight and polydispersity can be prepared via surface-initiated polymerization based on a number of controlled radical polymerization such as atom transfer radical polymerization (ATRP) and reversible addition-fragmentation chain transfer (RAFT).

Here in this research, we are interested to develop a substrate for SALDI-MS analysis based on surface-grafted poly(acrylic acid) (PAA) brushes containing AuNPs. Photolithography was first used to generate the hydrophobic/hydrophilic pattern to provide a visible contrast between the analyte spot and prevent cross-contamination of sample solution from one spot to another. PAA brushes were then grafted on

hydrophilic area of the pattern via surface-initiated RAFT polymerization of acrylic acid (AA). AuNPs were generated *in situ* within the PAA brushes by having carboxyl groups of PAA acting as reducing moieties for gold ions, without having to use an additional reducing agent. The substrate modified with PAA brushes containing AuNPs were then used for SALDI-MS analysis of a number of peptides, such as bradykinin which is a biomarker of breast cancer, thiol-containing peptides, namely glutathione and cysteine. It is anticipated that this developed patterned platform can be used for pre-concentration and separation of thiol-containing peptide from the peptide mixture in a high throughput fashion.

1.2 Objectives

1. To prepare and characterize the pattern of PAA brushes with *in situ* synthesized AuNPs
2. To study the usage of the modified substrate for bioseparation and detection of peptides by SALDI-MS

1.3 Scope of investigation

The stepwise investigation was carried out as follows:

1. Literature survey for related research work.
2. Preparation and characterization of the pattern on glass slide using photolithography.
3. To synthesize and characterize PAA brushes on patterned substrate
4. *In situ* synthesis of AuNPs
5. To study the potential of the modified substrate for bioseparation and detection by SALDI-MS.