CHAPTER 2

SPECTROSCOPY IN CARDIAC DIAGNOSIS

Highlights of the Chapter

- *Cardiac biomarkers and spectroscopic methods for its detection*
- *Spectroscopic detection methods developed till date*
- *Methods other than spectroscopic for biomarker detection are discussed.*

2.1 Cardiac Biomarkers

Evaluation of cardiac biomarker concentration provides important information about the status of heart[1], [2]. Many biomarkers had been identified and detection methods developed for their quantification. Use of biomarkers for detection of cardiac injury started way back. In 1954, La Due used aspartate aminotransferase for acute myocardial infarction[3]. Later, Creatine kinase and lactate dehydrogenase were reported as marker for cardiac abnormality. For a cardiac biomarker to fulfill requirements of an ideal biomarker, biomarker should be (a) Small molecule biomarkers as they reach circulation more rapidly (b) Cytosolic protein as they are released more rapidly than structural proteins after injury and so an earlier increase of level in plasma is obtained (c) Macromolecule of high solubility reaches plasma faster (d) Low local degradation of molecule after release as this may lead to low amount of detectable molecule than actually released due to injury and, (e) Specificity to myocardium[4]. Cardiac Troponins (I and T), C-reactive protein (CRP), Tumor necrosis factor-alpha (TNF- α), Myoglobin, and BNP/NT-proBNP are recently developed cardiac biomarker and generally used for cardiac health monitoring and diagnosis.

2.2 Spectroscopic methods

Phenomenon of inelastic scattering of monochromatic light when incident on a sample is Raman effect. The elastically scattered light known as the Rayleigh effect where light scatters with the same energy as incident light whereas inelastic light scatters with a different wavelength. This difference in energy of incident light and scattered light is called Raman shift which provides the fingerprint signature of the molecule[6].

Surface enhanced Raman spectroscopy is advanced method of Raman spectroscopy where signal intensity enhancement is observed. Intensity enhancement in SERS is now agreed to be dominantly contributed by electromagnetic enhancement mechanism. Chemical enhancement is also attributed to SERS intensity which is primarily based on the charge-transfer mechanism. Total SERS enhancement is the product of enhancement due to electromagnetic mechanism and chemical mechanism. In highly optimized systems, an enhancement by a factor of ~ 10^{10} to 10^{14} can be achieved[7].

Another spectroscopy method used in cardiac biomarker detection is fluorescence spectroscopy. In this technique, when an excitation light of constant wavelength and intensity interacts with a target molecule, resultant light is emitted by the molecule which is shifted in wavelength from excitation wavelength and is generally in visible light range. UV-Visible spectrophotometry has been also used in detection of several biomarkers. In this technique, absorption or reflectance of incident or excitation light is measured and change in intensity is directly related to target molecule concentration and interaction with excitation light.

2.3 Spectroscopy-based Troponin's detection

Cardiac troponins I (cTnI) and T (cTnT) are established as the most specific biomarker for detection of myocardial injury, risk stratification of patients, and diagnosis of myocardial

infarction. Cardiac biomarker detection assays primarily used gold nanoparticles to induce the SERS effect. Chon et al[8] used malachite green isothiocyanate or X-rhodamine-5-(and-6)-isothiocyanate coated hollow gold nanospheres to simultaneously detect cardiac troponin I and CKMB. Antibodies for capturing the target molecules troponins and CK-MB were immobilized on magnetic beads and target molecules were conjugated to the hollow gold nanospheres with Raman active dye. They claimed that their method doesn't require sample preparation and are very less influenced by sample dilution and matrix effects. Recently, Tu et al [9]developed an aptamer-based surface-enhanced Raman scattering assay on a paper fluidic platform for detection of cardiac troponin I. The group used gold nanoparticles functionalized with Raman reporter molecules which were encapsulated in a silica shell. They reported that the developed SERS assay had a detection range of 0.016 to 0.1 ng/mL. Jia et al[10] used zwitterionic peptides and aptamers for the construction of biosensors. The peptides used in the study were self-assembled on the gold chips and some of them were biotinylated. The developed biosensor showed a linear detection range from 20 ng/mL to 600 ng/mL with a limit of detection of 20 ng/mL. A few more recently developed SERS-based cardiac troponin detection biosensors are listed in Table 2.1.

Table 2.1: Cardiac troponin detection biosensors based on SERS

Biomarker	**Probe**	**Detection range**	**Detection limit**	**Reference (YOP)**
cTnI	DNA aptamer/silver layer	-	10 ng/ mL	[11](2020)
cTnI	Gold core with a silver shell	-	0.0044 ng/mL	[12](2020)
cTnI	Aptamer immobilized Au nanoplate	-	2.4 fg/mL	[13](2020)
cTnI	4MBA(Raman reporter molecule)	-	9.8 pg/mL	[14](2021)
cTnI	Aptamer	0-0.5 mg/mL	-	[9](2020)
cTnT	Silica shell encapsulated with Raman active molecule	10-100 pg/mL	10 pg/mL	[5](2021)
cTnI	CTAB stabilized AuNRs	0.5- 15 ng/mL	0.4 ng/mL	[15] (2021)

2.4 Spectroscopy -based CRP detection

C-reactive protein (CRP) is an emerging biomarker for early detection of cardiovascular diagnosis. It is a pentraxin protein i.e., acute phase protein produced by the liver. CRP level in patients with conditions like atherosclerosis and inflammation is observed to be high. Level of CRP increases about 1000 times in patients with acute inflammation.

Liu[16] group developed a lateral flow assay based on SERS using functionalized Fe_3O_4@Au magnetic nanoparticles. Target biomarkers were captured by nanotags and after washing, Fe_3O_4@Au nanotags acted as Raman reporter for quantification of target. The biosensor had a limit of detection of 0.01 ng/mL. In another study, Hu et al [12] designed an aptamer SERS based biosensor with reporter-labeled Au nano-bridged nanogaps particles and novel magnetic capture substrate. Aptamer against C-reactive protein was modified on both SERS tag and magnetic capture substrate for specific recognition by Au NNPs-CRP-Ag MNPs. The biosensor is found to have a limit of detection of 1.14 pg/mL. Wang et al[17] demonstrated a SERS based biosensor for detection of CRP using nanospheres and microcapsules. $CaCO_3$ first encapsulates rhodamine B to form microcubes and assemble layer by layer with poly (ether imide) and antibody. Functionalized magnetic Ni@C nanospheres were prepared to immobilize primary antibodies. Linear range for detection ranges from 0.1 pg/mL to 1 ug/mL with detection limit of 0.01 pg/mL.

Kim et al[18] developed label-free SERS detection of CRP on phosphocholine-terminated self-assembled monolayers. An amplified plasmon of concentration induced silver nanoparticles aggregates located ~4.0 nm away from CRP. The minimum detectable amount for the sensor was ~0.01 ng/mL in the buffer and ~0.1 ng/mL in 1% serum. Rong [19]group demonstrated a lateral flow assay using Raman reporter embedded gold-core silver shell nanoparticles with built-in hot

spots. The NPs were conjugated with the CRP detection antibody which acts as SERS tags. Limit of detection for the biosensor was 0.01 ng/mL.

2.5 Spectroscopy-based BNP/NT-proBNP and TNF- α detection

N-terminal pro B-type natriuretic peptides are small proteins that are either a hormone or part of peptide that is contained by a hormone. When the left ventricle faces difficulty to pump oxygenated blood to the body, BNP/NT-proBNP concentration level increases, reflecting that there may be a disease that affects either the heart or circulatory system. NT-proBNP is a better biomarker due to its longer half-life and higher stability in vitro. NT-proBNP is highly sensitive and specific for evaluation of cardiac evaluation.

He et al [20]demonstrated a SERS based immunosensor using metal-organic framework @ Au tetrapod's immobilized toluidine blue as SERS tag. Au nanoparticles functionalized $CoFe_2O_4$ magnetic nanospheres were prepared to assemble primary antibody. This acts as sandwiched antibody-antigen interaction amplifying the SERS signal. The biosensor was found to have a linear range from 1 fg/mL to 1 ng/mL with detection limit of 0.75 fg/mL. Su et al [21]introduced SERS based sandwich immunoassay using Raman reporter-molecule-labelled Ag-Au nano stars as nanotags and (3DOM)-Au-Ag-Au plasmonic array as substrate. The biosensors limit of detection was 0.41 fg/mL for NT-proBNP.

Tumor necrosis factor-alpha (TNF- α) is a pro-inflammatory cytokine produced during inflammation by macrophages for signaling events within cells. The normal concentration of TNF-α is stated to be in range 10 - 30 pg/mL. Highly sensitive and accurate detection biosensor is thus required to quantify TNF- α.

Gholami et al[22] developed SERS quenching nano sensor of quantification of TNF- α. They used benzothiazole azo dye as Raman probe which absorbs terminal group sulfhydryl (SH)

extracted from TNF- α by reduction. This causes the displacement of Raman reporter molecules on the substrate surface causing quenching of SERS signal proportional to concentration. The biosensor can detect TNF- α concentration of as low as 173 pg/L. Lai et al [23]demonstrated a biosensor using magnetic bead pull down assay using purified and highly SERS active small clusters of gold nanoparticles. The assay was found to have a limit of detection of 1 pg/mL with detection range of 1 pg/mL to 10ng/mL.

2.6 Spectroscopy-based myoglobin detection

Myoglobin is a non-enzyme protein specific to the heart and produced by blood and skeletal muscle whenever cardiac injury occurs. It is one of the most used biomarkers for diagnosis of acute myocardial infarction. It is a small protein (17.8 kDa) released in blood from one to three hour of cardiac event and concentration rises from a normal value of 30 to 90 ng/mL to as high as 900 ng/mL. Shorie group [24]developed a nanohybrid mediated substrate for SERS by assembly of gold nanoparticles on exfoliated nanosheets of tungsten disulfide to form plasmonic hotspots. The fabricated biosensor was read by a 532 nm laser and showed significant signal enhancement. Linear range for the system was from 10 fg/mL to 0.1 ug/mL. In another study, authors fabricated a SERS substrate of canonical anodic aluminum oxide templates with a regular array of nanotips and gold nanoparticles were distributed by plasma sputtering. Waleed et al [25]demonstrated a label-free SERS based biosensor consisting of 3D silver anisotropic nano-Pinetree array modified indium tin oxide substrate. Also, three Ag nanostructure modified ITO substrates were developed to select the highest SERS. The limit of detection of the developed biosensor was 10 ng/mL. Das group[26] fabricated nanostructures using electroplating and lithography techniques to obtain gold nanograin aggregate structures of diameter range 80 to 100 nm with interstitial gap of 10-30 nm.

Drop coating deposition Raman was used to obtain results using micro deposition of samples. Limit of detection claimed by the authors was in order of attomole (10^{-18}).

2.7 Other Cardiac biomarker detection methods

Electrochemical detection: Important factors for determining the efficacy of a biomarker detection system in the emergency are detection time and concentration of biomarkers within the clinically important period. Electrochemical detection can serve the purpose as it can provide portability, modularity, small sample volume, and small detection time. Electrochemical detection systems generally use potentiometric, amperometric or impedimetric techniques for quantification of the biomarker in focus. Potential or current change by the reaction between target and probe is used to measure the concentration of the target.

Xiong et al [27]developed electrochemical impedance spectroscopy (EIS) based immunosensor using anti-cTnI antibody and Ag nanoparticles. They reported detection range was 0.02 to 1 ug/ml with the limit of detection (LOD) of 0.001 ug/ml. Gomes group [28]developed amperometry based immunosensor using cTnI antibody and carbon nanotubes. They stated that the fabricated biosensor can detect cTnI concentrations from 0.1 to 1.0 ng/ml with LOD of 0.033 ng/ml. Lee et al[13] fabricated a biosensor using DNA and Au nanospikes on a printed circuit board. The detection level for the biosensor was found to be 1 pM in human serum.

Field effect transistor (FET)- based detection: Several methodologies have been developed for label-free and ultrasensitive detection of biomarkers. FET-based biosensors are the ones which serves the purpose of being a simple and rapid diagnostic method. FET based systems, in general, have a semiconductor channel material between two closely placed metal electrodes, and the channel material is functionalized to capture the target. High sensitivity is achieved using

nanomaterials like silicon nanowires, Carbon nanotubes, and Graphene. These nano materials provide a high surface to volume ratio compared to nano-structured electrodes.

Surface plasmon resonance-based detection: Surface plasmon resonance (SPR)-based biosensors is an optical detection system in which change in the refractive index of a material upon a metal surface is measured. SPR has advantages like real-time analysis of samples, label-free detection, and high sensitivity without requirement of any amplification method. The refractive index change occurs due to electron oscillation between a free electron and charged metal surface with surface plasmon effect when light is applied to the metal surface. SPR in conjunction with nanobiotechnology extends the application of SPR based systems from laboratory to point-of-care test (POCT) devices.

This chapter outlined the details and methods of cardiac biomarker detection, more specifically spectroscopy-based methods for cardiac diagnosis. Critical review of existing methods helped to understand how previously proposed method encounter the major challenges of this field. Further, the concepts have been utilized to meet the goals of the thesis.

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