NON-INVASIVE DETECTION OF TROPONIN I FOR HEART ATTACK PREVENTION

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INTRODUCTION

Regardless of precautions taken to prevent heart attacks, the fact remains that these are still the leading causes of death. According to the World Health Organization report, 59% of deaths were the result of non communal conditions. Of which, 29% of deaths were related to cardiovascular complications. This corresponds to approximately 10 million people in a year die resulting from cardiovascular complications.[1]

Most heart attacks could be maintained if the patient is to receive essential medical attention in time. Missed acute myocardial infarction (AMI) is the leading subject of medical malpractice claims against hospital emergency rooms and primary care. On the other hand admission of patients with a low probability of AMI often leads to excessive and unnecessary hospital costs.[2]

Cardiac biomarkers play an important role in determining the level of AMI risk. cTnI (Cardiac Troponin I) is a cardiac specific protein released into blood after inception of cardiac damage and is considered one of the most specific cardiac biomarkers. The purpose of this study is to design a portable real-time monitoring system for detection of heart attack at its early stages using non-invasive biophotonic measurements. For this purpose, Fluorescence, and Raman spectroscopy methods have been investigated. After a complete scan through cTnI sample using a Spectrofluorometer, fluorescence excitation wavelength of known concentrations of purified recombinant cTnI was found to be 283nm as expected due to existence of Tryptophan amino acid in the protein. The emission wavelength of protein for various molarities was acquired to be 347nm with elevated spectrum amplitude for higher protein concentrations. The regression graph of the concentration vs. fluorescence amplitude shows a 0.99 correlation, confirming linear relationship of cTnI fluorescence amplitude with respect to increase in concentration. A distinct Raman signature of cTnI sample diluted separately in Tn Buffer Complex and water have been acquired using Raman Spectrometer which demonstrates increase in amplitude of the Raman signature as molarities of the sample change.

SYSTEM OVERVIEW

Detectable levels of cTnI are reached in 3-6 hours after an AMI and will peak within 14 to 20 hours and return to normal after 5 to 7 days. cTnI concentration in normal serum is below 0.1 ng/ml and in AMI patients this levels increases to as high as 100–300 ng/ml.[3] The extraordinary cardiac specificity of cTnI solves the problem of other cardiac biomarkers false positive for conditions unrelated to AMI. According to large number of studies cTnI is the current “Gold Standard” test for diagnosis of AMI. Compared to other cardiac biomarkers (i.e. CK-MB and Myoglobin), cTnI has superior sensitivity, higher specificity and, more ability to detect minor heart muscle damages.[4]
The main purpose of this project is to investigate the design of a heart attack detection system which detects heart muscle damage shortly after the onset of AMI based on the increase of cTnI protein in the circulation. Also this project allows for investigation on feasibility of the purposed system.

The proposed system uses short pulses of UV light on the frontal vessels of the eye in order to measure direct backscattered fluorescence for differential measurement on cTnI. The pulse frequency will be set such that minimal damage is done on the cornea also the light source will be directed to the frontal vessels so that it causes as minimal damage as possible to the back nerves and vessels of the eye. The eye has been chosen for signal acquisition since there is more access to fresh blood and less scattering than other tissues.

EXPERIMENTAL PROCEDURES

A series of spectroscopy experiments and analysis were performed in order to find the most feasible non invasive cTnI detection method.

Fluorescence Spectroscopy

Owing to the presence of a Tryptophan (residue 191 shown in Figure 1), it can be expected that cTnI

protein will fluoresce. The double bonds of the conjugated systems will result in the absorption only in the UV region. Therefore Fluorescence Spectroscopy can be considered to be a valid detection tool for monitoring the concentration change of cTnI.

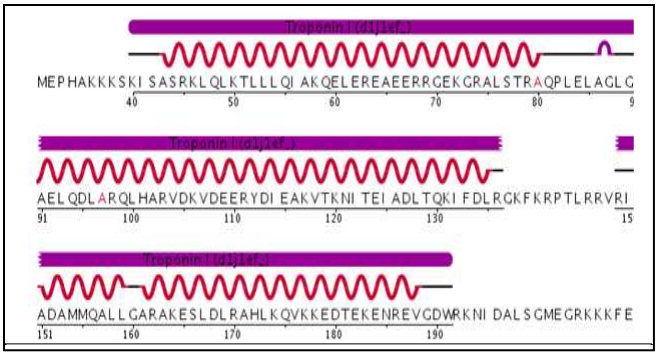

Figure 1: Human Cardiac Troponin I Amino Acid Sequence[5]

By acquiring a complete scan through the cTnI sample using PTI Quantamaster UV-Vis Spectrofluorometer, the excitation wavelength of the protein was found. As illustrated in Figure 2, the excitation wavelength of cTnI is 283 nm as expected from the existence of Tryptophan.

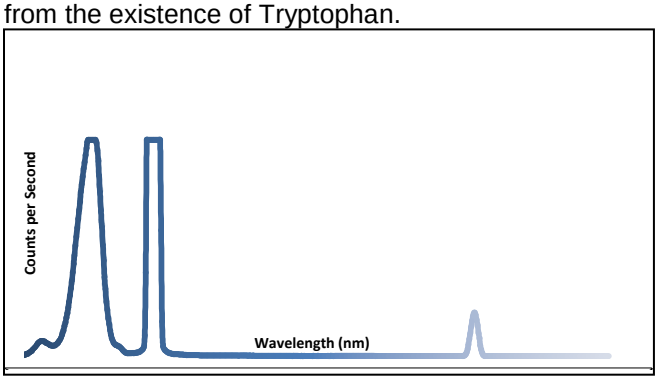

Figure 2: Scan through cTnI sample to find the excitation wavelength

The data graph of the emission of cTnI sample when excited at 280nm is shown in Figure 3. The extra peaks at higher frequencies are artifacts due to PTI Quantamaster Spectrofluorometer performance. The emission wavelength for different concentrations of cTnI in water happens at 347 nm as expected. The second peak which appears at higher wavelength is again caused by the device.

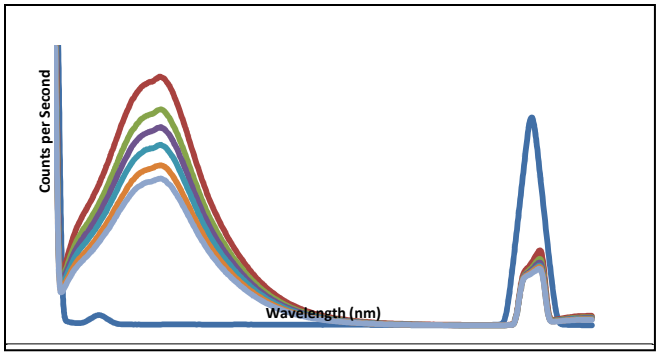

Figure 3: The Emission Graph at Different Concentrations when excited at 283nm

In order to monitor the fluorescent behaviour of the protein with an increase in concentration, a linear regression analysis was performed. From the regression analysis output, the slope of the line is 48252 counts/μM, the y-intercept is 529833.6 counts and the correlation is 0.95. The graph of the regression fit is presented in Figure 4. This gives an indication of the linear fluorescence characteristic of this protein with respect to increase in concentration within the experimental and human error.

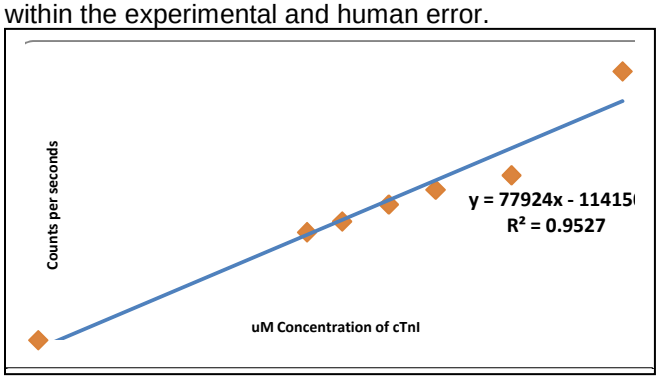

Figure 4: Linear Regression Graph of cTnI Concentration vs. Peak Signal Intensity

Raman Spectroscopy

The weakness of fluorescence spectroscopy for this application is the relative non specificity of cTnI signal in the blood which is not easily distinguishable from other conjugated proteins.

"Raman spectroscopy measures the exchange of energy with electromagnetic (EM) radiation of a particular wavelength. This method does not suffer from water interference as water is a very weak scatterer. Therefore Raman measurements can be made directly from biofluids and there are even reports of in vivo measurements."[6] Also the Raman response of biological substances is a more specific type of measurement comparing to fluorescence signal.

In order to investigate the possibility of cTnI detection using Raman spectroscopy, two experiments were designed and conducted. For the first experiment a commercialized SpectraCode RP-1 Portable Raman Spectrometer was used. Figure 5 presents the spectrum of water which was used as negative control for this experiment.

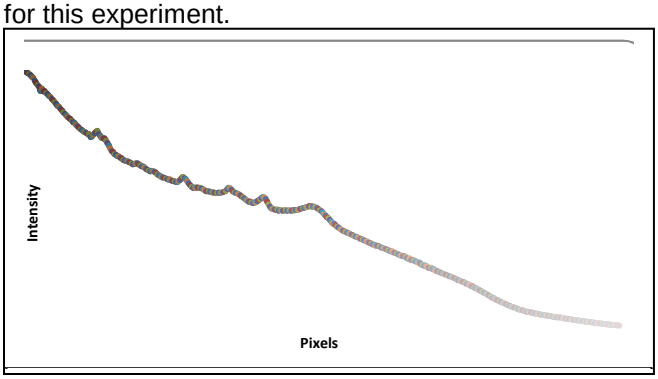

Figure 5: Raman Spectrum of water

5 µ gr of cTnI was diluted in 3mL of water and the concentration of the solution was decreased iteratively and spectrums were acquired (shown in Figure 6).

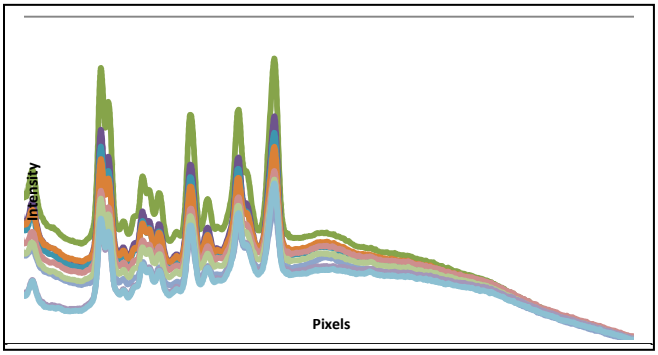

Figure 6: Raman Spectrum of cTnI in water

For the second experiment cTnI sample was prepared and diluted in Tn Complex Buffer. The initial concentration of cTnI in Tn Complex buffer was 2.47 mM. Tn Complex buffer was selected due to its similarity to Serum for the purpose of cTnI dilution to prepare different concentrations. For this experiment LABRAM Spectrometer was used. The instrument consists of four parts; an internal HeNe (633nm) laser, a microscope, a spectrometer: dispersing the Raman signal and optics for coupling the lasers to the sample, and carrying the Raman signal through to the spectrometer.

As expected from Figure 6 and Figure 7 it can be concluded that the Raman signal intensity of cTnI decreases in the water or Tn Complex as the sample is more diluted which makes Raman spectroscopy a promising method for detection of cTnI in the Serum.

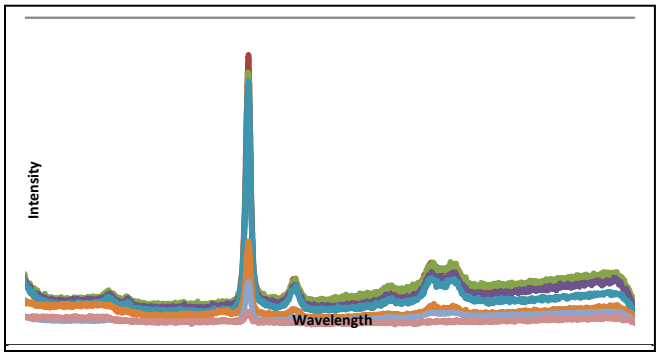

Figure 7: Raman Spectrum of cTnI in Tn Complex

CONCLUSION AND FUTURE WORK

In this project the fluorescence characteristics of cTnI protein was investigated and it was concluded that the data demonstrate a linear fit. The future work on this project will be performed on improvement of signal detection and also instrument miniaturization. Most importantly before any further investigation, a more complete feasibility study should be performed in order to select the most reliable and viable method for detection of cTnI. In order to extract a unique characteristic of the protein from its spectrum and to be able to distinguish cTnI protein from other proteins containing amino acids with conjugated properties, quantum effects should be considered. The high amount of spectral noise caused by blood content decreases the specificity of cTnI emission characteristic and method sensitivity. Also detection of backscattered signal instead of the transmitted signal have higher amplitude and would be more reliable.

Therefore the only anticipation which allows for further examination of this project is through efficiency in measuring small changes of the spectrum after protein concentration increases in the blood which leads to further examination of the Raman signature of the protein. Moreover a cTnI protein specific Raman spectrometer will be designed which will extract this protein spectral signature from much higher noise amplitude.

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