Swift Journals

Swift Journal of Medicine and Medical Sciences Vol2(2) pp. 011-024 April, 2016. http://www.swiftjournals.org/sjmms Copyright © 2016 Swift Journals

Original Research Article

Application of Fourier Transform Infrared (FTIR) Spectroscopy and Atomic Force Microscopy in Stroke-Affected Brain Tissue.

Mohamed. H. M. Ali¹, , Khalid Al-Saad², , Anton Popelka², Geralda van Tilborg³, Erik Goormaghtigh⁴.

¹(Qatar Biomedical Research Institute, QBRI, Doha, Qatar).

²(Qatar University, (Khalid Al-Saad, Depratment of Chemistry) and (Anton Popelka, Center for Advanced Materials, Doha, Qatar). ³Biomedical MR Imaging and Spectroscopy Group, Center for Image Sciences, University Medical Center Utrecht, Utrecht, the Netherlands.

⁴Université Libre de Bruxelles, Structure and Function of Biological Membranes Center for Structural Biology and Bioinformatics Brussels, Belgium.

Accepted 26th January, 2016.

ABSTRACT

Fourier transform infrared (FTIR) spectroscopy was assessed as a potential rapid and objective diagnostic platform to investigate pathological and physiological changes at 9 weeks post stroke. Atomic force microscopy (AFM) was also used in order to investigate the changes in bio-mechanical and bio-physical properties of the brain due to stroke. Unilateral stroke in the sensorimotor area was induced by photothrombosis in rats, under 1.5-2% isoflurane anesthesia. Brains were extracted, post-fixated in 4% paraformaldehyde and embedded in paraffin. FTIR spectra of the stroke-affected samples showed that there is a decrease in the white matter lipid content. The FTIR results indicated that there are bio-chemical changes in amide I band in the protein region as well as in the ester band. Principal component analysis (PCA) in the 1800-1000 cm-1 spectral range showed that the white matter in stroke-affected brain sample exhibits chemical composition changes in an amide I, amide II and ester bands. AFM results implied that physical changes in the stroke samples led to the significant increase of the surface roughness as the result of increased tension in the brain tissue. The changes in the surface morphology resulted in the increase of mechanical parameters such as elastic modulus (Er) and hardness (H) of stroke samples. FTIR spectroscopy in combination with chemometric analysis a non-destructive and powerful approach to be used as a medical diagnosis tool in order to investigate the pathological changes associated with stroke in the brain tissues. The AFM confirmed that the surface roughness increase in stroke samples.

Keywords: FTIR, Brain stroke, PCA, AFM, Surface morphology and Nanoindentation

BACKGROUND

Infrared (IR) spectroscopy is a one-photon effect and the photon absorption results in a vibrational motion of a molecule. Infrared spectra originate from the vibrational motions of atoms in chemical bonds within the molecular structure (Theophanides T. 1984). When a beam of light containing the (IR) radiation interacts with a sample, IR energy is absorbed by the chemical bonds causing the vibrational motions. Each molecule absorbs radiation at a specific frequency will produce

a spectral IR band at the corresponding wavenumber (cm-1) (Theophanides T. 1984). The IR spectra vibrational frequencies provide information about specific structures of bio-molecules and produce a specific biochemical and biophysical fingerprint of the sample (Downes A. 2010) (Srinivasan G. 2010). Fourier transform infrared (FTIR) spectroscopy is a powerful analytical and diagnostic technique and has been widely applied to the characterization of different

*Corresponding Author: Mohamed. H. M. Ali, Qatar Biomedical Research Institute, QBRI, Doha, Qatar *E-mail:* moaam@yahoo.com tissues bio-chemical makeup due to its sensitivity to the chemical and architecture and information about the molecule (Vasiliki D. 2012). Molecular vibrations within biological samples typically produce broad, overlapping, and complex IR bands which originate from numerous individual components that often have the same chemical functionality. However, the IR spectra are mainly dominated by biochemical building blocks markers such as proteins, lipids, carbohydrates and nucleic acids (Carter EA. 2007). Changes in the ratios, positions and height of these diagnostic band markers are attributed to bio-chemical and structural changes in the biological system.

These biological changes depend on growth status, cell cycle or disease state (Shaw RA. 2000) (Naumann D. 2000) in complex biological systems, the IR spectrum provides a wealth of information about protein and/or lipid conformational and structural changes (peak position and line-shape), or increases/decreases in the amount of a specific bio-chemical makeup within a biological sample (peak intensity) (Carter EA. 2007). Stroke is a loss of brain function(s) when the blood supply of the brain is suddenly interrupted or when the brain blood vessel is bursting. Stroke is the main cause of adult disability such as permanently paralyzed on one side of their body or lose their ability to speak. In 2020, stroke will account for 6.2% of the total burden of illness (Meairs S. 2006). Improve prevention, treatment and rehabilitation of stroke will ease a huge social and economical burden. Ischemic stroke is the main stroke subtype and accounting for about 87% of all stroke cases (www.strokassociation.org).

Currently, there is an increase in the field of research to investigate the pathological and physiological changes due to stroke. Interestingly, it has been found that after the acute neurological deficits, most stroke patients improve and exhibit a certain degree of recovery. Several studies showed that the gradual restoration of functions is attributed to structural and functional modifications in surviving brain tissue. The modifications such as the modulation of neurotransmission, unmasking or strengthening of existing neural pathways, and formation of new neuronal connections can play a role in brain function recovery (Murphy TH. 2009) (Nys J. 2015) (Ganguly K. 2013). The cellular and molecular mechanisms underlie the structural and functional modifications of neural networks is not completely understood yet.

The current work will investigate the bio-chemical composition of the white and grey matter as well as biochemical changes in white matter at 9 weeks after stroke in rats. Fourier transform infrared (FTIR) spectroscopy is an accurate, sensitive and rapid diagnostic technique. The FTIR spectroscopic technique is simple, reproducible, nondestructive, and does not need tedious sample preparation [4]. FTIR spectroscopy has been used in cancer diagnosis such as breast (Fabian H. 2002) (Fabian H. 2006) (Tam KM. 2005) (Rehman IU 2010) (Jacek KP. 2009) (Gao T. 1999) (Tolstorozhev GB. 2010) (Ooi GJ. 2008) (Ci Y. 1999) (Dukor RK. 1998), colon (Rigas B. 1992) (Yao H. 2014), prostate (Worden KL. 1999), brain (Jackson M. 1995) (McIntosh LM. 1999) (Malins DC. 1996) (Gajjar K. 2013) (Stelling AL. 2013) (Eckel R. 2001) (Meurens M. 1996), cervix (Wood BR. 2006) (Wood BR. 2004) (Wood BR. 1998) (Romeo MJ. 2002) and also, individual normal and cancerous cells (Levin IW. 2005) and other organs (Afanasyeva NI. 1995) (Hrebičík M. 1997).

The results showed that FTIR spectroscopy could differentiate malignant lesions from normal and benign tissues. FTIR spectroscopy has already been used extensively to analyze nerve tissues in order to understand the biochemical and structural changes associated with neurodegenerative

diseases. FT-IR spectroscopy has been applied to investigate different neurological disease such as Alzheimer's, Parkinson's, transmissible spongiform encephalopathies, multiple sclerosis, as well as brain tumors (Choo LP. 1996) (Kneipp J. 2000) (LeVine SM. 1993) (Choo LP. 1993) (Lewis EN. 1996). The aim of this study is to use the FTIR spectroscopy in order to investigate the biochemical changes in the white matter of rat's brain that is associated with the stroke after nine weeks.

MATERIALS AND METHODS

Photothrombotic stroke and tissue processing 11-weeks old male Sprague Dawley rats were purchased from Charles River (Germany). Rats were anesthetized with 4% isoflurane in air/O2 (2/1), followed by maintenance at 1.5-2% isoflurane in air/O2 (2/1). Rats received an endotracheal tube for mechanical ventilation, before they were placed in a stereotaxic frame. Blood oxygenation and heart rate were continuously monitored (Nonin Medical) during surgery and body temperature was maintained at 37±0.5 °C. Experimental stroke was induced by photothrombosis (Pennekamp CWA. 2012).

The skin was opened, periosteum was removed and the skull was dried. Next, the illumination area was defined between +4mm to -4mm anterior/posterior and 1.5 mm to 4.5 mm lateral to bregma, using black tape. Subsequently, an optic fiber, mounted on a cold light source (Schott KL 1500 LCD, Germany) and equipped with a green fluorescent filter (28 mm, 515 nm; Schott), was placed over the sensororimotor cortex on the intact skull. Prior to illumination, the photosensitive dye Rose Bengal (Sigma) was infused through the vena saphena (25mg/kg) at an infusion rate of 5.625 mg/min.

FTIR Spectroscopy

FTIR spectra were obtained using FTIR spectrometer at a reflection mode within the range of 4000-700 cm⁻¹. FTIR images were recorded using a focal plane array instrument with a 64x64 Mercury Cadmium Telluride (MCT) focal plane array detector. The images were obtained at reflection mode in the range of 4000-700 cm⁻¹ with 128 scans per spectrum, at a 2.7 x 2.7 μ m2 spatial resolution and 4 cm⁻¹ spectral resolution. Spectral image analysis was subsequently performed in Matlab 6.1 (The Mathworks Inc.) after preprocessing to remove spectra that having high noise to signal ratio and to remove baseline and scattering effects.

FT-IR images (using raw absorbance and/or absorbance ratios) were constructed for initial analysis of molecular concentration and type. The spectra were processed using the following: a) Gaussian apodization: 4 cm⁻¹; b) Flattening CO2

Swift. J.Med.Medical.Sc. | 013

between 2450 and 2250 $\rm cm^{-1};$ c) Savitzky-Golay method; d) Vector normalization between 1800 and 1000 $\rm cm^{-1}.$ Principal component analysis (PCA) was performed on the processed

data in the spectral range of 1800-1000 $\rm cm^{-1}$ using Matlab software.



Figure 1. FTIR spectra collected from white matter in healthy brain tissue in the spectral range of 4000 -1000 cm⁻¹.



Figure 2. FTIR spectra collected from grey matter in healthy brain tissue in the spectral range of 4000-1000 cm⁻¹.



Figure 3. FTIR spectrum collected from white matter in stroke-affected brain tissue in the spectral range of 4000-1000 cm⁻¹.

The PCA was calculated using [V,L]= eig (cov(x)) to get the eigen vector of the covariance matrix of the data stored in x. The current work has been done on three (paraffin-embedded

formalin fixed, (PEFF) blocks of healthy control samples and eight stroke-affected brain samples 9 weeks after photothrombotic stroke. The measurements were performed on five sections from each block. The spectra represented in the work is one of hundreds spectra that were collected from the brain sections.

The collected spectra from the healthy and stroke-affected brain samples are reproducible and repeatable for both healthy and stroke-affected brain samples. Surface morphology analyses 3D Optical Surface Metrology System Leica DCM8 was used for a visualization of prepared brain tissue samples. Non-destructive confocal technology can be applied for the measurement of the sample surface with complex shapes or steep inclinations of up to 70°. The high sensitive detector with high resolution (1.4 million pixels) allows viewing of the confocal images. Comprehensive surface data with a high contrast are quickly captured. The images were obtained by measurements of 323 and 336 topographies in control and stroke samples using EPI 20X-L objective.

The information about the surface morphology of brain tissue samples were obtained by an Atomic Force Microscopy (AFM). The AFM device MFP-3D Asylum research (USA) equipped with a Silicon probe (AI reflex coated Veeco model – OLTESPA, Olympus) was used in our experiments. Measurements were performed under ambient conditions using the Standard Topography AC air (tapping mode in air). An AFM head scanner applied with Si cantilever adjacent vertically in the sample resonant frequency of the freeoscillating cantilever set as the driving frequency. The selected areas for representative AFM measurements were carefully chosen for individual AFM measurement after a previous screening of the surface regularity.

Nanoindentation

The information about nano-mechanical properties of brain tissue samples were obtained using nanoindentation technique. The MFP-3D NanoIndener (Asylum Research, USA) with Standard tip indenter with spring constant 3940N.m-1 (calibration with Sapphire standards) was used. The three-sided Berkovich diamond indenter tip composed of an industrial diamond brazed to a screw-threaded hex tooled metallic chuck was used in our experiments. Results about nano-mechanical properties were evaluated from 4 different areas (10 indentations in each). The indentation measurement procedure was consisted of three steps: 1. Loading force from 0 to 50μ N at 10μ N/s of loading rate; 2. indentation force was kept under constant value for 5s and 3. unloading from 50 to 0μ N at 10μ N/s of unloading rate.

The data were analyzed from unloading curves according to an Oliver-Pharr model for an elastic half space deformation by an elastic punch relating to the contact area at peak load to the elastic modulus. The hardness (H) of brain tissue samples was determined by the H=Fmax./A, where A is the projected area and Fmax is maximum applied load. The reduced modulus (Er) of brain samples obtained from this measurement is expressed by 1/Er=(1-v2)/E+(1-vi2)/Ei) in which "v" Poisson's ratio of sample, "E" Young's modulus of sample, "vi" Poisson's ratio of indenter (0.2) and "Ei" Young's modulus of indenter.

RESULTS

The results showed that different areas of the brain samples, according to their biochemical makeup, had different patterns of the FTIR spectra. In general, the spectra obtained from each area were highly reproducible and had good signal-to-noise ratio. Certain spectral bands such as protein lipid and ester bands were selected as bio-markers to identify the different tissues in the brain sample. Constructing FTIR images based on these bands were used as a vital approach in differentiating between the different tissues.

Figures 1 and 2 show the FTIR spectra collected from the healthy rat brain white and grey matter, respectively. The white and grey matters spectra of the brain sections showed five spectral regions as shown in Figures 1 and 2, respectively. The spectra were dominated by the protein, lipid and ester regions. The protein region appeared in the range of 1800-1480 cm⁻¹. This region was dominated by the amide I (~1695-1637 cm⁻¹) and II (1543 cm⁻¹) bands that arose from C=O stretching and N-H bending vibrations of the peptide bonds (Helm D. 1991). Fatty acids (lipids) region was in the range 3000-2750 cm⁻¹.

This region was dominated by the two bands at 2920cm^{-1} (uas(CH2)) and at 2850 cm-1 (us(CH2)) that arose mainly from C-H stretching vibrations of the CH2 functional groups of fatty acid chains of the cell membrane and side groups of polypeptide chains in proteins (Helm D. 1991). The ester region found at ~ 1730 cm⁻¹ was assigned to the C=O stretching vibration of lipid ester functional groups (Maquelin K. 2002). It can be observed from Figure 1 that the white matter spectrum had two stronger absorption bands at 2920 and at 2850 cm⁻¹ when compared to those from the grey matter (Figure 2). These bands arose from C-H stretching vibrations of –CH3 and –CH2 of the fatty acid content of the white matter.

The results in Figure 1 also showed that the protein/lipid ratio in the white matter spectrum was lower compared to protein/lipid ratio in the grey matter (in Figure 2). The spectra also showed that there was slightly stronger absorption in the ester region at ~ 1730 cm^{-1} for the white matter. The results indicated that the bio-chemical makeup of the white matter and the grey matters was different and the white matter was richer in lipid than the grey matter. Figure 3 shows the FTIR spectrum of the white matter ipsilesional (same side of the stroke location) in a brain section obtained at 9 weeks after photothrombotic stroke. A slight decrease in the C-H stretching absorption bands were observed, suggesting degradation of chains or a decrease of the relative amount of fatty acid.

The FTIR spectrum in Figure 3 also shows that the band ratio of amide l/amide II slightly increased compared to the spectrum of the control sample (Figure 1). The ester absorption band at ~ 1730 cm^{-1} became less distinctive and weaker compared to the spectrum collected from the normal brain section. This result may indicate that the white matter in the induced stroke brain section lost some of its lipid content.

FTIR spectroscopic results showed that FTIR is a powerful technique to follow lipid changes through the specific absorbance bands that arise from the ester carbonyl group, methyl and methylene groups (Coates J. 2000) (Ismail AA. 1997) (Orsini F. 2002) (Maguelin K. 2002).

Figures 4a and 4b shows an overlay of the microscopic and FTIR images, respectively, in the white matter of healthy rat brain tissue. Figure 4c represents the spectra collected at different locations, as indicated by the colored regions of interest in Figure 4b. In the fatty acid region at the spectral range of 3000-2750 cm⁻¹, the collected spectra exhibited strong bands at 2920 cm⁻¹ and 2850 cm⁻¹ that arose from C-H stretching vibrations. These bands arose from lipids, phospholipids of cell membranes. Figures 5a and 5b show the microscopic and FTIR images, respectively, collected from ipsilesional white matter stroke-affected rat brain tissue. Figure 5c shows the FTIR spectra at different points of the white matter in the induced stroke brain section. The spectra show that the absorption bands arising at 2920 and 2850 cm⁻¹ were weaker compared to those in the control brain sample (in Figure 4). The result also showed that there were spectral

changes in the amide I band that arose at (1680-1620 cm-1). The spectra in Figure 5c also indicate that there was a decrease in the C=O band at about 1730 cm⁻¹ which arose from ester. Figure 6 shows FTIR images from ipsi- (left) and contra (right)-lesional white matter of stroke-affected brain

tissue sections from five different animals at 9 weeks post stroke.



Figure 4. FTIR imaging and spectra of the white matter in a healthy rat brain tissue. Spectra were collected in the range of 4000-1000 cm⁻¹. 4a) the microscopic section of the brain control sample; 4b) the FTIR image of the microscopic section and 4c) The FTIR spectra from different locations indicated on the FTIR image in 4b.



Figure 5. FTIR imaging and spectra of the white matter of stroke-affected brain sample. The spectra were collected in the range of 4000-1000 cm⁻¹. 5a) the microscopic section of the white matter in the same side (ipsilesional) of the stroke-affected brain; 5b) the FTIR image of the microscopic section of the white matter in the same side (Ipsilesional) of the induced stroke and c) The FTIR spectra from different locations indicated on the FTIR image in 5b.

Figure 7 shows the second derivative of the collected FTIR spectra from the images in Figure 6 in the range of 1800-1000 cm-1. The range of interest of 1800-1000 cm⁻¹ was chosen because it exhibits the varying characteristics in the spectra. The second derivative spectra were processed in order to perform the chemometric analysis and extract the principal components analysis from the spectra at specific bands and regions.

The second derivative spectra in Figure 7 were collected under the same experimental and measurement conditions in the range of 1800-1000 cm⁻¹. The following spectral differences were observed: a) in the ester region in the spectral range around ~ 1730 cm¹, there was a spectral change between the spectra collected from the right (contralesional) and left side (ipsilesional) of the brain sections; b) in the protein region in the range of 1700-1500 cm¹, amide I band at (~1695-1637cm⁻¹) experienced a spectral shift between the spectra and c) there were spectral and concentration differences between the amide I and II bands collected from the right (contralesional) and left side (ipsilesional) of the brain sections. Principal component analysis (PCA) could not differentiate between the spectra of the stroke-affected brain sections when the full spectral range was included in the analyses.

Second derivatives of the spectra were used in the 1800-1000 cm⁻¹ spectral range, where differences were most apparent among the spectra collected from the five induced stroke brain sections on both sides. The spectra could be differentiated based on their IR spectral data, as shown in Figure 8 (a, b, c, d, e and f). PC1 in Figure 8a describes the major spectral differences between both sides of the same section, where the induced stroke occurred in only one side and PC1 accounts for 42.9% of the total spectral variation. These differences are shown in Figure 9a (blue spectrum), which depicts the loading spectrum of PC1. The differences were mainly located in the regions of amide I band at (~1695-1637 cm⁻¹) and there was a small variation in the amide II band at (1543 cm⁻¹).

These bands arose from C=O stretching and N-H bending vibrations of the peptide bonds. Figure 8b shows the PC2, which accounts for 21.4% of the overall spectral variation among the spectral data. These variations are shown in Figure 9a (green spectrum), which represents the loading spectrum of the PC2. The variations were principally located in the regions of amide I band at (~1695-1637 cm⁻¹) and small variation in the amide II band at (1543 cm⁻¹). The loading spectrum of the PC2 also shows that there was spectral variation in the ester region at ~ 1730 cm⁻¹. Figure 8c shows the PC3, which accounts for 7.6% of the overall spectral variations among the spectral data. These variations are shown in Figure 9a (red spectrum), which represents the loading spectrum of the PC3. Figures 8 (d, e and f) show PC4, PC5 and PC6 and account for 5% and 3.2% and 2.9%, respectively. The loading spectra of the PC4, PC5 and PC6 are shown in Figure 9b. The variations principally were located in the protein region, mainly amide I band at (\sim 1695-1637cm⁻¹) and ester band at about 1730 cm⁻¹. The variations in the spectral data and chemometric spectral analyses indicate that the biochemical composition of the white matter may experience some changes due to the stroke.

Surface Morphology Analyses

Non-destructive confocal 3D Optical Surface Metrology System was used for a visualization of prepared brain tissue samples on the glass sheet. The 3D images of healthy and strokeaffected brain samples are shown in Figure 10. The prepared samples excelled regularly structures in the surface area. Detailed information about the surface morphology of brain tissue samples was analyzed from AFM measurements. This technique allows obtaining 3D images with high resolution. Moreover, the information about surface roughness represented by an average deviation (Ra) was obtained. The Ra, also known as an arithmetic average, is defined as the average absolute deviation of the roughness irregularities from the mean line over one sampling length. The 3D images and Ra values of healthy and stroke-affected brain tissue samples are shown in Figure 11. The healthy brain sample showed characteristic surface morphology in 10x10µm area with relative low roughness.

On the other hand, physical changes in the surface morphology of stroke-affected brain sample are clearly seen leading to a significant increase of the Ra value as a result of increased tension in the tissue. Analyses of surface morphology in $1x1\mu$ m area provided a detailed view on the surface structures of brain tissue samples. The healthy sample was characterized by indented surface structures. In case of stroke-affected brain samples, the small indents disappeared and relative smooth surface irregularities was observed, probably because of the stretching process in the tissue.

Ra = 99.6nm A Ra = 154.3nm B Ra = 20.6nm C Ra = 40.3nm DFigure 11: AFM

Figure 11: AFM images of brain tissue samples at $10x10\mu m$ area: A - Healthy, B - Stroke-affected brain; and at $1x1\mu m$ area: C - Healthy, D - Stroke-affected brain.

Mechanical Properties Investigation

The information about the mechanical properties of brain tissue samples was obtained using nanoindentation technique. This technique allows to measure indentation depths of thin samples in the surface area by the application of very low forces to the indentation tip. The Er and H average values of healthy and stroke-affected brain samples are summarized in Table 1 and the representative loading and unloading curves are shown in Figure 12. The average values of Er and H of the stroke-affected brain samples increased by about 65% and 78%, respectively. The increase of mechanical properties of the stroke-affected brain samples are caused probably by straightens processes in the tissue, leading to the loss of flexibility.

DISCUSSION

In this study, the chemical composition and molecular changes in the control and induced stroke rat's brain paraffin embedded sections after three weeks have been investigated using FTIR spectroscopic imaging technique.

Furthermore, the biochemical makeup composition and differences between the white and gray matter have been studied. Infrared spectroscopy allows for the study of the lipid, protein, ester and the functional group content of tissue sections with minimal sample preparation (Choo LP. 1993) (Lewis EN. 1996) (Helm D. 1991). The content of the sample remains unaltered, providing information that cannot be gained by any other method.



Figure 6. Ten FTIR images collected from ispi- (left column) and contra-(right column) lesional white matter from stroke-affected brain sections from five different animals obtained at nine weeks after stroke.



Figure 7. Second derivatives FTIR spectra used to build the images reported in Figure 6 (white matter in both sides from the five stroke-affected brain tissue sections obtained at 9 weeks after stroke) in the 1800-1000 cm⁻¹ range.

www.swiftjournals.org

Also, the technique is a non-destructive, sensitive and a fast tool with no labels, stains or dyes required, compared to conventional histo-pathological diagnostics techniques (Maquelin K. 2002) (Ismail AA. 1997) (Orsini F. 2002).

The FTIR images and spectroscopic results are in good agreement with other works that showed that the brain white matter consists mostly of myelinated axons that contain high lipid content veined with capillaries (Gough KM. 2012). This result was represented by strong absorption bands of the white matter in the lipid and phospholipid region in the range of 3000-2750 cm⁻¹. The results also revealed that brain gray matter contains less lipid content, which agrees with other published work that stated that the brain gray matter is made up of neural cell bodies, neuropil, glial cells, and capillaries and so it contains very little lipid (Kansiz M. 2014). The spectral features of the stroke-affected samples indicated that there was a decrease in the C-H lipid bands which could be related to lipid oxidation in the white matter after a stroke.

Protein configuration changes were also noticed in the stroke tissue samples and represented mainly in the amide I band at (~1695-1637 cm⁻¹). The FTIR images collected from the stroke-affected brain side showed that the brain stroke might cause changes in the lipid, ester and protein configuration and concentration in comparison to the healthy brain tissue. Principle component analysis (PCA) in the 1800-1000 cm-1 spectral range showed that there was chemical composition and concentration changes mainly located in an amide I band at (~1695-1637 cm⁻¹), amide II band at (1543 cm⁻¹) and ester band (C=O) at (1730 cm⁻¹). Results showed that the reduced elastic modulus (Er) and hardness (H) of the

stroke-affected brain samples increased significantly (by about 65% and 78%, respectively) when compare with healthy brain samples.

The results indicate that brain tissue characteristics experienced bio-mechanical and bio-physical alterations due to stroke. The stroke-affected brain samples ultrastructural showed that there is a permanent and prominent cytoskeletal structure deformation as was indicated by the mechanical properties studies of the brain tissues. The AFM technique is a valuable technique for surface tracking of the cell shape, integrity, and roughness changes in the brain tissues affected by stroke. We conclude that the combined AFM and nanoindentation analyses of brain tissues could give thorough and valuable information about the brain disease status and the degree of the brain damage. This information is significant for the brain disease medical management after experience stroke.

The results showed that FTIR imaging spectroscopy in contribution with chemometric analysismodalities provided a detailed chemical image from which chemical information can be extracted. FTIR spectroscopic imaging is a valuable technique which enables the study of basic physiology and disease processes, as it monitors changes in the biochemical makeup, distribution and concentration of biological molecules (Burklen TS. 2006) (Chwiej J. 2010) (Heraud P. 2010). In summary, FTIR imaging spectroscopy has shown significant promise as a powerful technique for medical diagnosis and it offers unique opportunities for molecular assessment of brain tissue plasticity.

Principal component analysis 1800-1000 cm⁻¹



Figure 8: (a, b, c). The first 3 principle components analysis of the FTIR images collected in Figure 6, in the range 1800-1000 cm⁻¹.



Figure 9a: Loading spectra of the PC1 (42.9%), PC2 (21.4%) and PC3 (7.6%) shown in Figure 8 (a, b and c) in the range of 1800-1000 cm⁻¹ for of the FTIR images collected in Figure 6.



Figure 9b. Loading spectra of the PC4 (5.0%), PC5 (3.2%) and PC6 (2.9%) shown in Figure 8 (d, e and f) in the range of $1800-1400 \text{ cm}^{-1}$ for of the FTIR images collected in Figure 6.



Figure 10: 3D images of brain tissue samples obtained by 3D Optical Surface Metrology system: A – Healthy and B - Stroke-affected brain.

Sample	Er (GPa)	SD (GPa)	H (MPa)	SD (MPa)
Healthy	1.99	0.52	83.55	12.55
Stroke	3.29	0.72	148.44	21.19

Table 1: Mechanical properties of healthy and stroke-affected brain samples.

SD - standard deviation



Figure 11: AFM images of brain tissue samples at 10x10µm area: A - Healthy, B - Strokeaffected brain; and at 1x1µm area: C - Healthy, D - Stroke-affected brain.



Figure 12: Representative loading and unloading indentation forces of brain samples: a – Healthy and b - Stroke-affected brain.

Mid- IR spectroscopy is more frequently used in medical applications of infrared research, using radiation in the region of 4000–400 cm⁻¹ (Carter EA. 2007) (Naumann D. 2000) (Maquelin K. 2002).

Wavenumber (cm–1)	Assignment	
3328	Amide A: N–H and O–H stretching vibrations of polysaccharides, proteins	
3129	Amide B: N–H stretching vibrations of proteins	
3015	Olefinic =CH stretching: unsaturated lipids, cholesterol esters	
2960	CH3 antisymmetric stretching: lipids, protein side chains	
2920	CH2 antisymmetric stretching: mainly lipids	
2875	CH3 symmetric stretching: protein side chains, lipids	
2850	CH2 symmetric stretching: mainly lipids	
1720–1745	C=O stretching vibrations of lipids (triglycerides and cholesterol esters)	
1710–1716	C=O antisymmetric stretching: RNA and purine base	
1705–1690	C=O antisymmetric stretching vibrations: RNA, DNA	
1654	Amide I: C=O (80%) and C–N (10%) stretching, N–H (10%)	
	bending vibrations: proteins a-helix	
1630–1640	Amide I: C=O (80%) and C–N (10%) stretching, N–H (10%)	
	bending vibrations: proteins β-structure	
1610, 1578	C4-C5 and C=N stretching in imidazole ring of DNA, RNA	
	1515 Aromatic tyrosine ring	
1540–1550	Amide II: N–H (60%) bending and C–N (40%) stretching vibrations:	
	proteins α-helix	
1530	Amide II: N–H (60%) bending and C–N (40%) stretching vibrations:	
	proteins β-structure	
1467	CH2 bending vibrations: lipids and proteins	
1455	CH3 bending and CH2 scissoring vibrations: lipids and proteins	
1370–1400	COO- symmetric stretching and CH3 bending vibrations: lipids,	
	proteins	
1330-1200	Amide III: proteins	
1230–1244	PO2- antisymmetric stretching vibrations: RNA, DNA and phospholipids	
1090-1080	PO2- symmetric stretching vibrations: RNA, DNA	
1060, 1050	C–O stretching vibrations: deoxyribose/ribose DNA, RNA	
996	RNA stretch and bend ring of uracil	
965	PO4– symmetric stretch (DNA) and deoxyribose-phosphate skeletal motions	
925–929	Sugar vibrations in backbone of DNA-Z	

ACKNOWLEDGMENTS

This work was made possible by a NPRP award [NPRP5 – 381 - 3 - 101] from the Qatar National Research Fund (a member of The Qatar Foundation). The statements made herein are solely the responsibility of the authors.

REFERENCES

- 1-Theophanides T. Fourier Transform Infrared Spectroscopy. D. Reidel Publishing Co., Dodrecht, 1984.
- 2- Downes A, Mouras R, Elfick A. Optical spectroscopy for noninvasive monitoring of stem cell differentiation. J. Biomed. Biotechnol. 2010; 2: 1-10.
- 3-Srinivasan G. Vibrational Spectroscopic Imaging for Biomedical Applications, the McGraw-Hill Companies, 2010.
- 4-Vasiliki D. FTIR Spectroscopy in Medicine, Infrared Spectroscopy -Life and Biomedical Sciences, Prof. Theophanides Theophile (Ed.), ISBN: 978-953-51-0538-1, InTech, Available from: http://www.intechopen.com/books/infrared-spectroscopy-life-andbiomedical-sciences/FTIR-spectroscopy-inmedicine. 2012.
- 5-Carter EA, Marshall CP, Ali MHM, Ganendren R, Sorrell TC, Wright YC. Lee, Chen CI, and Lay PA. Infrared Spectroscopy of Microorganisms: Characterization, Identification, and Differentiation. Downloaded by UNIV OF SYDNEY on June 14, 2011 | http://pubs.acs.org Publication Date: August 2, 2007 | doi: 10.1021/bk-2007-0963.ch006. In New Approaches in Biomedical Spectroscopy; Kneipp, K., et al.; ACS Symposium Series; American Chemical Society: Washington, DC, 2007.
- 6-Shaw RA, Mantsch HH. In Encyclopedia of Analytical Chemistry; Meyers, R. A., Ed. John Wiley & Sons Ltd.: Chichester, UK, 2000; 1:83-102.
- 7-Naumann D. In Encyclopedia of Analytical Chemistry; Meyers, R. A., Ed. John Wiley & Sons Ltd.: Chichester, UK, 2000; 1:102-131.
- 8-Meairs S, Wahlgren N, Dirnagl U, Lindvall O, Rothwell P, et al. Stroke Research Priorities for the Next Decade - A Representative View of the European Scientific Community: Summary of Stroke Research Workshop, Brussels, October 25, Cerebrovasc. Dis. 2006; 22.2-3:75-82.
- 9-http://www.strokeassociation.org.
- Murphy TH, Corbett D. Plasticity during stroke recovery: from synapse to behavior. Nat. Rev. Neurosci. 2009; 10:861-872. doi:10.1038/nrn2735. 11- Nys J, Scheyltjens I, Arckens L. Visual system plasticity in mammals: the story of monocular enucleationinduced vision loss. Front Syst. Neurosci. 2015; 9: 60. doi: 10.3389/fnsys.2015.00060. 12- Ganguly K, Poo M-m. Activity-Dependent Neural Plasticity from Bench to Bedside. Neuron 2013; 80 (3):729–741.
- 13-Fabian H, Lasch P, Boese M, Haensch W. Mid-IR microspectroscopic imaging of breast tumor tissue sections. Biospectroscopy 2002; 67: 354-357.
- 14-Fabian H, Lasch P, Boese M, Haensch W. Diagnosing benign and malignant lesions in breast tissue sections by using IRmicrospectroscopy. Biochimica et Biophysica Acta (BBA) -Biomembranes. 2006; 1758 (7):874-882
- 15-Tam KM, Carter EA, Lay PA, Armstrong RS, Mountford CE, Dowd S, Russell P, Lee YC, Chen YL. Characterization of Ductal Carcinoma in-Situ Breast Cancer Using IR Micro-spectroscopy, 6th ACOVS, The University of Sydney 2005; 39-40.
- 16-Rehman IU, Rehman S, Movasaghi Z, Darr JA. Fourier Transform Infrared Spectroscopic Analysis of Breast Cancer Tissues; Identifying Differences between Normal Breast, Invasive Ductal Carcinoma, and Ductal Carcinoma In Situ of the Breast. Applied Spectroscopy Reviews. 2010; 45:355–368.
- 17-Jacek KP, Achim K, Ying Y, Paul D, Sirinart C-S, Michel M, Ganesh DS, Josep S-S. Spectroscopic signatures of single, isolated cancer cell nuclei using synchrotron infrared microscopy. Analyst 2009; 134:1176-1181.
- 18-Gao T, Ci Y. FTIR spectroscopic assessment of water structure in human breast benign and malignant tissues. Anal. Commun. 1999; 36: 341-343.

- 19-Tolstorozhev GB, Skornyakov IV, Butra VA. IR spectroscopic methods for diagnosis of oncological pathologies in human tissues (Review). Journal of Applied Spectroscopy 2010; 76 (6): 761-771.
- 20-Ooi GJ, Fox J, Siu K, Lewis R, Bambery KR, Don McNaughton, Wood BR. Fourier transform infrared imaging and small angle xray scattering as a combined biomolecular approach to diagnosis of breast cancer. Med. Phys. 2008; 35: 2151. http://dx.doi.org/10.1118/1.2890391.
- 21-Ci Y, Gao T, Feng J, Guo Z. Fourier transform infrared spectroscopic characterization of human breast tissue: implications for breast cancer diagnosis. Appl. Spectrosc. 1999; 53: 312-315.
- 22-Dukor RK, Liebman MN, Johnson BL. A new, non-destructive method for analysis of clinical samples with FTIR microspectroscopy. Breast cancer tissue as an example. Cell. Mol. Biol. 1998; 44: 211217.
- 23-Rigas B, Wong PTT. Human Colon Adenocarcinoma Cell Lines Display Infrared Spectroscopic Features of Malignant Colon Tissues. CANCER RESEARCH 1992; 52: 84-88.
- 24-Yao H, Shi X, Zhang Y. The Use of FTIR-ATR Spectrometry for Evaluation of Surgical Resection Margin in Colorectal Cancer: A Pilot Study of 56 Samples, J. Spectroscopy 2014; http://dx.doi.org/10.1155/2014/213890
- 25-Worden KL, Song Y, Mason RP, Liu H. Measurement of Tumor Oximetry: a Comparison between Near-infrared Frequency-Domain Spectroscopy and 19F NMR Spectroscopy of Hexafluorobenzene. Proc. SPIE-Int. Soc. Opt. Eng. 1999; 3597: 601-610.
- 26-Jackson M, Choo L, Watson PH, Halliday WC. Beware of connective tissue proteins: assignment and implications of collagen absorptions in infrared spectra of human tissues, Biochim. Biophys. Acta 1995; 1270: 1-6.
- 27-McIntosh LM, M. Jackson, H. H. Mantsch, M. F. Stranc, D. Pilavdzic, A. N. Crowson Infrared spectra of basal cell carcinomas are distinct from non-tumor-bearing skin components. J. Invest. Dermatol. 1999; 112: 951-956.
- 28-Malins DC, Polissar N L, Gunselman SJ. Progression of human breast cancers to the metastatic state is linked to hydroxyl radicalinduced DNA damage. Proc Natl Acad Sci U S A. 1996; 93(6): 2557–2563.
- 29-Gajjar K. et al. Diagnostic segregation of human brain tumours using Fourier-transform infrared and/or Raman spectroscopy coupled with discriminant analysis. Anal. Methods 5,89–102 (2013).
- 30-Stelling AL, Toher D, Uckermann O, Tavkin J, Leipnitz E, Schweizer J, Cramm H, Steiner G, Geiger KD, Kirsch M. Infrared Spectroscopic Studies of Cells and Tissues: Triple Helix Proteins as a Potential Biomarker for Tumors. PLOS ONE | www.plosone.org. 2013; 8 (3).
- 31-Eckel R, Huo H, Guan H-W, Hu X, Che X, Huang W.-D. Characteristic infrared spectroscopic patterns in the protein bands of human breast cancer tissue. Vib. Spectrosc. 2001; 27: 165-173.
- 32-Meurens M, Wallon J, Tong J, Noel H, Haot J. Breast cancer detection by Fourier transform infrared spectrometry. Vib. Spectrosc. 1996; 10 (2): 341-346.
- 33-Wood BR, Bambery KR, Evans CJ, Quinn MA, Don McNaughton. A three-dimensional multivariate image processing technique for the analysis of FTIR spectroscopic images of multiple tissue sections. BMC Med. Imaging 2006; 6(1):12-24.
- 34-Wood BR, Chiriboga L, Yee H, Quinn MA, McNaughton D, Diem M. Fourier transform infrared (FTIR) spectral mapping of the cervical transformation zone, and dysplastic squamous epithelium. Gynecol. Oncol. 2004; 93 (1):59-68.
- 35-Wood BR, Quinn MA, Tait B, Ashdown M, Hislop T, Romeo M, McNaughton D. FTIR microspectroscopic study of cell types and potential confounding variables in screening for cervical malignancies. Biospectroscopy 1998; 4 (2):75-91.
- 36-Romeo MJ, Quinn MA, Burden FR, McNaughton D. Influence of benign cellular changes in diagnosis of cervical cancer using IR microspectroscopy. Biopolymers 2002; 67: 362–366.
- 37-Levin IW, Bhargava R. FOURIER TRANSFORM INFRARED VIBRATIONAL SPECTROSCOPIC IMAGING: Integrating Microscopy and Molecular Recognition. Annu. Rev. Phys. Chem. 2005; 56: 429-474.

- 38-Afanasyeva NI, Artjushenko V, Kolyakov S, Letokhov V, Lerman A, Nikolaev G, Sokolov V. MIR fiber spectroscopy for minimal invasive diagnostics. Proc. SPIE-Int. SOC. Opt. Eng. 1995; 2627: 228-230.
- 39-Hrebičík M, Budínová G, Godarská T, Vláčil D, Vogenseh SB, Volka K. Diffuse-reflectance infrared Fourier transform spectroscopy: new technique of sample preparation. MOL. STRUCT. 1997; 410–411:527–530.
- 40-Choo LP, Wetzel DL, Halliday WC, Jackson M, LeVine SM, Mantsch HH. In situ characterization of beta-amyloid in Alzheimer's diseased tissue by synchrotron Fourier transform infrared microspectroscopy, Biophys. J. 1996; 71: 1672–1679.
- 41- Kneipp J, Lasch P, Baldauf E, Beekes M, Naumann D. Detection of pathological molecular alterations in scrapie-infected hamster brain by Fourier transform infrared (FTIR) spectroscopy, Biochim. Biophys. Acta. 2000; 1501: 189–199.
- 42-LeVine SM, Wetzel DLB. Analysis of brain tissue by FTIR microspectroscopy, Appl. Spectrosc. Rev. 1993; 28: 385–412.
- 43-Choo LP, Jackson M, Halliday WC, Mantsch HH. Infrared spectroscopic characterisation of multiple sclerosis plaques in the human central nervous system, Biochim. Biophys. Acta. 1993; 1182: 333–337.
- 44-Lewis EN, Gorbach AM, Marcott C, Levin IW. High-fidelity Fourier transform infrared spectroscopic imaging of primate brain tissue, Appl. Spectrosc. Rev. 1996; 50: 263–269.
- 45-Pennekamp CWA. Carotid revascularization and cerebral perfusion monitoring: clinical & experimental studies. 2012; ISBN: 978-90-393-5997-6. 46- Helm D, Labischinski H, Naumann D. Elaboration of a procedure for identification of bacteria using Fourier-transform IR spectral libraries: a stepwise correlation approach. J. Microbiol. Meth. 1991; 14:127-142.
- 47-Maquelin K, Choo-Smith LP, Kirschner C, Ngo-Thi NA, Naumann D, Puppels GJ. Vibrational spectroscopic studies of microorganisms. John Wiley & Sons Ltd. New York, 2002; 1-40.
- 48- Coates J. Interpretation of Infrared Spectra, A Practical Approach. in Encyclopedia of Analytical Chemistry, R.A. Meyers (Ed.) John Wiley & Sons Ltd, Chichester, 2000; 10815–10837.
- 49-Ismail AA, van de Voort, FR, Sedman J. Fourier Transform Infrared Spectroscopy: Principles and Applications. In Instrumental Methods in Food Analysis. J.R.J. Paré and J.M.R Bélanger, Eds. Elsevier Science B.V. 1997; 93-139.
- 50-Orsini F, Ami D, Villa AM, Sala G, Bellotti MG, Doglia SM. FTIR microspectroscopy for microbiological studies. J. Microbiol. Methods. (2002); 42:17-27.
- 51-Maquelin K, Kirschner C, Choo-Smith LP, van den Braak N, Endtz HP, Naumann D, Puppels GJ. Identification of medically relevant microorganisms by vibrational spectroscopy. J. Microbiol. Methods 51 (2002) 255-271.
- 52-Gough KM, Kuzyk A, Kirkwood J. Rapid screening of brain tissue with a high spatial resolution over large analysis areas using Agilent's 670 and 620 FTIR imaging analysis. 2012;1-4.
- 53-Kansiz M, Hirschmugl C, Albensi B, Liao C, Gough K. High Spatial resolution FTIR imaging of biomedical tissue samples using a novel method of magnification enhancement. 2014;1-6.
- 54- Burklen TS, Schlattner U, Homayouni R, Gough K, Rak M, Szeghalmi A, Wallimann T. The creatine kinase/creatine connection to Alzheimer's disease: CK inactivation, APP-CK complexes, and focal creatine deposits. J. Biomed. Biotechnol. 2006, 1–11.
- 55-Chwiej J, Dulinska J, Janeczko K, Dumas P, Eichert D, Dudala J, Setkowicz Z. Synchrotron FTIR microspectroscopy study of the rat hippocampal formation after pilocarpineevoked seizures. J. Chem. Neuroanat. 2010; 40, 140–147.
- 56-Heraud P, Caine S, Campanale N, Karnezis T, McNaughton D, Wood B R, Tobin M, Bernard CCA. Early Detection of the Chemical Changes Occurring During the Induction and Prevention of Autoimmune-mediated Demyelination Detected by FTIR Imaging. NeuroImage 2010; 49:1180–1189.