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The use of vibrational spectroscopy to study the pathogenesis of multiple sclerosis and other neurological conditions

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Abstract

Spectroscopy techniques are valuable tools in biomedical research and have been used extensively in the study of disease. However, neurological conditions such as multiple sclerosis (MS) have received little attention and the available spectroscopy studies are limited, both in overall numbers of patients studied and the disease samples considered. MS is a complex immune-mediated disease, with variable clinical courses and limited therapeutic options. This review aims to summarize current literature in the area, demonstrating how spectroscopy techniques can provide valuable information to inform and advance research into the most common neurological condition affecting young adults.
Introduction

Biophotonic techniques are now widely used in biomedical research targeting better diagnosis, prognosis and surveillance of disease. Vibrational spectroscopy methods such as Fourier-transform infrared (FTIR) and Raman spectroscopy are so called because they probe the intramolecular vibrations and rotations of a sample when irradiated with a light source (1). The vibrational energy levels can be probed by both techniques, using different physical processes. Raman spectroscopy studies the Raman effect, the spontaneous inelastic light scattering process of photons, following the interaction of monochromatic radiation (e.g. a laser) with the sample. In contrast, FTIR spectroscopy studies the samples’ absorption characteristics arising from the molecular motion due to atomic displacement upon interaction with an infrared source (2, 3). In both cases, the recording of vibrational energy level transactions results in a spectrum composed of peaks/bands that can be interpreted qualitatively (peak position) and quantitatively (peak intensity/area) (4). In FTIR spectroscopy, the spectral bands arise from a change in the electric dipole moment of the molecules, whereas in Raman spectroscopy, they arise from a change in molecular polarizability. FTIR and Raman spectroscopies are therefore complementary and provide a “fingerprint” or “signature” of the specific molecules contained within a biological sample (proteins, lipids, DNA), depending upon whether their bonds exhibit infrared or Raman activities. Both FTIR and Raman can be used for imaging tissue sections and are non-destructive, label-free techniques with sub-micron spatial resolution (5).

In biomedical research, scientists are continually investigating and exploring the application of new technologies that can detect early signs of disease and thereby reduce disease morbidity and mortality. The detection of biomarkers plays an important role in this exploration. In oncology, such biomarkers, have been used extensively to determine risk factors, aid diagnosis and prognosis, and in the assessment of treatment response as well as determining disease recurrence (4). However, from amongst the vast numbers of candidate biomarkers, only a limited few have been validated for clinical use. Vibrational spectroscopy is new investigatory tool in biomarker (re)search which is not restricted to the analysis of a specific protein, nucleic acid and/or lipid. As such, FTIR and Raman spectra are able to give spectral "signatures" or "biomarkers" which reflect the overall molecular composition of the studied samples (4).

Despite being extensively used in the field of cancer research (6, 7), FTIR and Raman spectroscopy are currently under explored in the study of diseases which affect the central nervous system (CNS) including multiple sclerosis (MS). To date, there are very few published papers in this field including a review article published in 2012 (8).
Multiple Sclerosis (MS)

MS is considered to be an autoimmune, neuro-inflammatory and degenerative condition, which affects both the brain and spinal cord. Its precise aetiology remains unknown, although both genetic and environmental factors influence an individual’s susceptibility to develop MS (9). The clinical course of this disease is variable but is divided into several categories reflecting the degree of clinical disease activity and disability progression rate, including relapsing remitting (RRMS), primary progressive (PPMS) and secondary progressive MS (SPMS) (10). Whilst the inflammatory component of MS pathogenesis is relatively well understood, the progressive neurodegenerative component of the disease, in both its primary and secondary progressive clinical courses, is yet to be elucidated. In PPMS, patients have a gradual and progressive decline in function from the outset, with minimal disease activity detectable on magnetic resonance imaging (MRI) whereas in SPMS, the gradual progression follows an initial relapsing remitting phase, usually over many years (11). The consensus is that MS is a spectrum of conditions with RRMS being one end of that spectrum and PPMS being at the other.

The key pathological features observed in MS are the influx of inflammatory immune cells across the blood brain barrier into the CNS which results in the loss of axons and their insulating myelin sheaths and the formation of lesions (plaques) in the white matter (WM) and to a lesser extent in the grey matter (12). This process results in the impairment of conduction along the affected axon leading to variable symptoms experienced by affected patients including cognitive impairment, visual disturbances, sensory and motor symptoms, impaired balance, sphincter disturbance and fatigue (13). Histopathological comparisons of CNS tissue shows that the classical perivascular inflammation seen in SPMS is much less prominent in PPMS and that more diffuse inflammatory changes and greater extent of axonal damage in the normal appearing white matter (NAWM) are seen in PPMS (14, 15). In addition, there is evidence that patients with PPMS have a reduced capacity for remyelination (11). Understanding the underlying pathogenesis which underpins the clinical progression in MS at the molecular and cellular levels is therefore vital for the development of therapies targeting the neurodegenerative process and enhancing remyelination strategies.

The diagnosis of MS is usually based on the clinical presentation and the results of brain and spinal MRI, which reveals evidence of active and chronic lesions as well as focal and generalised atrophy (16). Current treatments for MS target the initial relapsing phase of the disease, by preventing inflammatory responses leading to a reduction in the number and severity of relapses (17). However, there are currently no treatments for primary and
secondary progressive MS although two therapeutic agents are waiting to be licenced (18). 

The underlying pathogenesis of the initial inflammatory phase of MS has been well 
characterised at both the cellular and molecular level. However the pathogenesis of the 
progressive phase is still not fully elucidated, although changes in the NAWM appear to be 
pivotal (12). The progressive loss of axons seem to continues despite the reduction of 
relapses with the use of effective anti-inflammatory therapies resulting in irreversible 
disability which support the presence of two separate pathological processes: inflammation 
and neurodegeneration (19).

Approaches to studying MS pathogenesis have focussed on the analysis of post-mortem 
CNS tissue, as well as experimental work using both primary CNS cells such as astrocytes, 
microglia and oligodendrocytes isolated from CNS tissue, which allows manipulation of the 
individual cell's environment. In addition, a number of animal models of MS have been 
developed, primarily in rodents but also in primates, in order to investigate the disease 
course of MS. In these animals, experimental autoimmune encephalomyelitis (EAE) is 
induced through the injection of spinal cord homogenates, myelin proteins or peptides, such 
as myelin oligodendrocyte glycoprotein (MOG), myelin basic protein (MBP) or proteolipid 
protein (PLP), in addition to adjuvant. This promotes the induction of an autoimmune 
response against myelin, leading to both an inflammatory response in the CNS as well as, 
dependent on the model used, demyelination (20). Nevertheless, the progressive aspects of 
the human condition seen in MS are more difficult to reproduce in animal models (21), 
although Peferoen has recently described an EAE model in Biozzi mice which, dependent on 
the age of the mouse at induction of disease, demonstrates progressive disease, with 
younger mice having an initial relapsing remitting phase followed by a secondary 
progressive phase and older mice showing progression at onset of disease induction (22). A 
further drawback of these animal models is that off the multitude of therapies found to be 
effective in preventing EAE, a very small number have been taken forward into clinical trials 
(23), suggesting that the models do not fully mimic the pathogenesis of MS in humans.

Current research approaches to investigating the aetiology of MS focus on the search for 
specific genes/proteins/lipids that are thought to be involved in the disease process using a 
variety of cell and molecular biology approaches. More recently DNA microarrays have been 
used to assess more global changes in gene expression in MS diseased human CNS tissue 
compared with normal age matched control (24, 25). The application of proteomic and 
metabolomic analyses in MS have focussed on biomarkers in biological fluids, including 
blood, cerebrospinal fluid and urine rather than in MS CNS tissue (26, 27).
The advantage of Raman and FTIR for analysis of human tissues is that the overall chemical composition of the tissue in terms of lipids, nucleic acids and proteins is obtained. Spectroscopic study of MS has been reported in the literature using both human post-mortem CNS tissue and animal models of the disease. A review of all available original research articles published to date is provided below and is summarized in Table 1.

**Human CNS tissue spectroscopy studies**

Initial studies applying spectroscopic techniques to the study of MS pathology were reported in the 1990s. Choo et al. were the first to use FTIR to study human white and grey matter tissue, obtained from healthy control subjects, and compared it with MS demyelinated lesions tissue from MS patients (28). This rapid communication reported that it was possible to discriminate between different types of MS tissue attributed to variations in intrinsic lipid and water content. Whilst FTIR spectra of white matter was dominated by lipids and protein absorptions and grey matter spectra showed reduced lipid content alongside an increase contribution of water to the spectra; MS lesion spectra were suggestive of both lipid and water depletion, as would be expected from histopathological tissue analysis (28).

Differences comparing white and grey matter as well as white matter with MS lesions were most notable in the 2800-3000 cm\(^{-1}\) spectral region, where most infrared bands arise from CH\(_2\) and CH\(_3\) stretching vibrations of lipid acyl chains. Four main assignments were made to CH\(_3\) and CH\(_2\) asymmetric stretching vibrations at 2956 and 2922 cm\(^{-1}\) and, to symmetric vibrations at 2871 and 2851 cm\(^{-1}\) respectively. The overall intensity of these peaks were reduced in both grey matter and MS lesion tissue, compared to normal control white matter, which the authors explained was due to the expected lower lipid content of grey matter and MS lesions, due to demyelination. Similarly, the CH\(_2\)/CH\(_3\) ratio is also decreased as a decrease in lipid to protein ratio leads to methylene and methyl groups of amino-acid side chains dominating this spectral region; as the CH\(_2\)/CH\(_3\) ratio in proteins is much lower than in lipids, it was expected that the overall ratio would be decreased and band broadening would be observed. In order to distinguish between grey matter and MS lesion tissue, the authors reported the spectral region of 1200-1800 cm\(^{-1}\) to be most useful (28).

The main feature in white matter spectra was observed at 1467 cm\(^{-1}\) and assigned to the scissoring vibration of CH\(_2\) groups of lipid acyl chains. In grey matter, the intensity absorption of this CH\(_2\) scissoring is reduced and is almost equal to the CH\(_3\) asymmetric bending vibrations at 1456 cm\(^{-1}\). This is explained by the reduction in lipid content, which is also
apparent by the decrease in intensity of the terminal methyl groups of lipid chains and of the 
(CH$_3$)$_3$N$^+$ symmetric bending of phosphatidylcholine headgroups, assigned to the bands at 
1381 and 1415 cm$^{-1}$, respectively. In contrast the COO$^-$ symmetric stretching band at 1400 
cm$^{-1}$ is increased in the spectra of grey matter in comparison to white matter. The same was 
also observed at 1308 cm$^{-1}$ which the authors assigned to amide III(28).

The spectral features of MS plaque tissue are similar to the ones described for grey matter. 
Nevertheless, the intensity of CH$_2$ scissoring (1467 cm$^{-1}$) to CH$_3$ asymmetric bending 
vibrations (1456 cm$^{-1}$) is now reversed with CH$_3$ asymmetric bending vibrations being the 
main feature in this region. Similarly, the PO$_2^-$ antisymmetric stretching band also displays 
greater intensity in the plaques' spectra comparatively to grey matter. Both these 
observations were suggested to indicate that lipid content of MS plaques is lower than that of 
the grey matter (28), which is known to be the case from histopathology studies (13).

In the 1500 to 11800 cm$^{-1}$ spectral range the main feature observed was the amide I band 
which arises from the C=O stretching vibration of amide groups of proteins and is centred at 
1653 cm$^{-1}$. Other absorptions reported were assigned to C-C stretching of tyrosine at 1517 
cm$^{-1}$; the amide II band centred at 1550 cm$^{-1}$; and the acidic amino-acid and arginine side 
chains at 1581 and 1580-1610 cm$^{-1}$ respectively. In addition, the ratio of amide I to amide II 
was increased comparatively to that of isolated proteins. It was suggested this may result 
from non-protein contributions to the amide I region, and further proposing water to be the 
main source of this contribution (28).

Le Vine et al. (1998) assessed active lesions in MS tissue compared with healthy control 
white matter and reported an increased oxidation state of both lipids and proteins in MS 
lesions, indicative of a role for free radicals in MS pathogenesis (29). The spectra of WM 
tissue from control post-mortem cases was dominated by CH$_2$ absorptions at 2923 and 1468 
cm$^{-1}$, P=O at 1235 cm$^{-1}$ and HO-C-H at 1060 cm$^{-1}$, characteristic of lipids, phospholipids and 
glycolipids respectively. Areas of NAWM within MS cases were reported to display similar 
spectra to normal control white matter, whereas lesion areas display significant changes, 
such as a reduced ratio of CH$_2$ to NH and OH, in comparison with control white matter. 
Further differences were revealed by the investigation of the oxidation products of lipids and 
proteins. Previous studies reported the amide I peak at ~1660 cm$^{-1}$ to be broader when 
proteins are oxidised and the carbonyl absorption at 1740 cm$^{-1}$ to be increased when lipids 
are oxidised. This study reported the C=O (1740 cm$^{-1}$) to CH$_2$ (1468 cm$^{-1}$) ratio to be 
increased and the peak at 1657 cm$^{-1}$ to be broader in MS lesions in comparison with white 
matter from control samples (29).
Furthermore, the authors followed the spatial spectroscopic profiles of these features by recording linear maps acquired partially or wholly within MS lesions sites and representative areas of control white matter. They reported the CH$_2$ (1468 cm$^{-1}$) to amide II (1544 cm$^{-1}$) ratio to be 0.644±0.053 for control samples (n=5), ranging from less than 0.1 to 0.7 in MS ones (n=5); 15.950±1.593 was the mean of the C=O (1740 cm$^{-1}$) to amide II (1544 cm$^{-1}$) ratio in control cases, which was in turn decreased for all MS cases; and four out of five MS cases presented one or more values above the mean of 24.047±3.22 for C=O (1740 cm$^{-1}$) to CH$_2$ (1468 cm$^{-1}$) ratio of control samples. Finally, whilst controls displayed an average of -0.033±0.010 at 1652 cm$^{-1}$, MS cases displayed greater values all above -0.02 (29).

It was concluded that the higher carbonyl to CH$_2$ ratio detected in the spectra of MS cases is suggestive of lipids being oxidised, whilst oxidation of proteins cause the 1657 cm$^{-1}$ peak to broaden to 1652 cm$^{-1}$ in MS plaque tissue. This result may be caused by gliosis, which occurs in parallel with the demyelination process leading to higher expression of glial fibrillary acidic protein by astrocytes, which was also indicated as a potential factor contributing to the amide I broadening, as well as the relative greater expression of amide II (29).

More recently, Poon et al. used Coherent Anti-Stokes Raman Scattering (CARS) to study several regions of post-mortem MS brain, including areas of NAWM, remyelination and both active and chronic lesions (30). Investigating five chronic MS cases, they reported a novel instrument that allows acquisition of high resolution, label-free imaging whose pixels contain spectral information, together with a post-processing method, which allows isolation and quantification of these spectral images. The study showed the CH$_2$ symmetric stretch of 2850 cm$^{-1}$ in NAWM, to shift to 2885 cm$^{-1}$ when myelin was contained within the phagocytic macrophages/microglia cells within the tissue (a CARS image is overlaid with immunostaining with the marker HLA-DR/LN3, confirming activated microglia). This was proposed to arise from the intermolecular chain disorder resulting from the breakdown of the myelin components during demyelination. Further CARS pseudo-colour images showed myelinated axons to have greatly reduced density within remyelinated areas in active lesion sites (30).

An additional study, also by Poon et al. reported lipid biochemical changes preceding myelin protein loss in peri-lesional areas and NAWM, when inspecting the CH spectral region from 2750 to 3100 cm$^{-1}$ (31). CARS images were acquired from the NAWM region adjacent to the lesion and sequential images were acquired moving away from the lesion into the NAWM. Triplicate images were also acquired from an area furthest away from the lesion site, referred to as "true NAWM" and from matched brain regions in tissue sections from control
non-MS cases. The average "true NAWM" spectra did not overlap with region-matched control spectra, suggesting possible underlying pathology in MS tissue, which is not differentiated when using lipophilic histochemistry or immunostaining with conventional techniques (31).

The three major features in the CH spectral region analysed, correspond to the symmetric and asymmetric and asymmetric stretching of acyl chain methylene at 2850 and 2886 cm\(^{-1}\) respectively, and the CH\(_3\) methyl chain end symmetric stretch at 2935 cm\(^{-1}\), which is thought to include protein contributions as well. Observing the intensity ratios of 2850/2880 cm\(^{-1}\) and 2935/2880 cm\(^{-1}\) the authors noted a slowing decreasing trend across all measured intensity ratios, when moving away from the lesion site until reaching the "true NAWM" ratios, recorded from an area the furthest away from the lesion, and approaching the ratios of region-matched non-MS control samples. The 2850/2880 cm\(^{-1}\) ratio is thought to relate to the intermolecular packing, interchain interactions and intrachain torsional motions, whereas the 2935/2880 cm\(^{-1}\) ratio allows monitoring intramolecular chain disorder and trans-gauche isomerisation. The authors conclude that biochemistry of myelin lipid content changes in the lesion periphery and in NAWM (31).

**Mouse models of demyelination and remyelination**

Animal models of MS have also been investigated by vibrational spectroscopy, where most studies aim to elucidate the mechanisms behind demyelination and remyelination. Heraud et al. used FTIR spectroscopy to investigate macromolecular components and protein conformational changes in the CNS of EAE versus control tissue sections (32). Using principal component analysis (PCA) and artificial neuronal networks (ANN) to analyse single data acquisition spectra, the authors demonstrated, without the need for chemical stains, subtle chemical and structural changes, particularly in the secondary structure of proteins in the white matter (33).

Fu et al. used resonant CARS imaging from the symmetric CH\(_2\) stretch vibration at 2840 cm\(^{-1}\) to characterize myelin changes induced by lysophosphatidyl choline (lyso-PtCho) (34). Although not directly relevant to demyelinating diseases including MS, the authors reported CARS was able to characterise the changes occurring in lyso-PtdCho-induced myelin breakdown and that together with electrophysiological data, it revealed involvement of a Ca\(^{2+}\), calpain, and cPLA\(_2\)-dependent pathway (34).

In another study, CARS was used to study myelin loss in the mouse-model, Relapsing-EAE (R-EAE) (35). Two theories have been hypothesised for initiating demyelination, one where
the injury starts at internodal myelin, thinning layer by layer and the other, where it initiates with paranodal domain injury. The authors noted that the submicron spatial resolution of CARS images allowed not only the quantification of myelin thickness but also the ratio of myelin thickness to the axonal diameter at different stages of the disease process. Furthermore, two-photon immunofluorescence microscopy revealed that juxtaparanodal $\mathbf{K}^+$ channels, paranodal myelin retraction and the displacement of $\mathbf{K}^+$ channels was extensively observed at the onset of R-EAE and at lesion borders. Overall their results suggested loss of nodal integrity precedes the formation of myelin debris in the CD4$^+$ T-cell-mediated R-EAE model of MS and that remyelination is accompanied by reestablishment of the nodal makers, with myelin being only partially restored (35).

Furthermore, the Raman spectra of myelin were dominated by lipid assignments and the authors studied both C-C and C-H vibrational bands to determine the conformation of their hydrocarbon chains through: (1) lipid packing studied using prominent bands at 2850, 2885 and 2930 cm$^{-1}$, assigned respectively to stretching and asymmetric stretching of $\mathbf{CH}_2$ and to $\mathbf{CH}_3$ stretching; and, (2) lipid unsaturation using the $\mathbf{I}_{1650}/\mathbf{I}_{1445}$ ratio, which represents the $\mathbf{C}=\mathbf{C}$ stretching bands to H-C-H deformation bands in lipid acyl chains. Myelin debris presented a higher intensity of the $\mathbf{I}_{2930}/\mathbf{I}_{2885}$ ratio, reflecting an increased intermolecular chain disorder; and regenerated myelin presented a higher lipid-packing disorder than normal myelin. Similarly, myelin debris presented the highest unsaturation degree, which was decreased in regenerated myelin but nevertheless was higher than normal myelin. Finally, the analysis of the $\mathbf{I}_{1122}/\mathbf{I}_{1076}$ ratio, revealed no significant change could be observed in the intramolecular chain ordering of myelin debris, normal and regenerated myelin (35).

A non-invasive multimodal CARS system, combining reflectance for visualizing axons, fluorescence to visualize green fluorescence protein (GFP) and Raman to visualize myelin and to monitor microglia induced neurodegeneration was reported by Imitola et al. (36). Using an EAE model, the authors reported fast ex vivo imaging of myelin, axons and microglia with great anatomical precision in live tissue. CARS images showed a global decrease in myelination, not seen before through other imaging techniques. This suggests that subtle alterations in the myelin lipid content may precede hallmark CNS demyelination, which is correlated with axonal loss and microglia activation (36).

Wang et al. reported DBT (3,3'-diethylthiatricarbocyanine iodide) to be a promising probe for Near Infrared Fluorescence (NIRF) imaging of myelination (37). Through in vivo NIRF studies on hyper and hypomyelination mouse models, the authors demonstrated DBT successfully enters the brain and selectively binds to myelin sheaths. Furthermore, aiming to
broaden NIRF-DBT imaging to MS disease, the authors studied a cuprizone-induced mouse
model for demyelination and remyelination. NIRF imaging and quantitative analysis revealed
DBT could successfully monitor the level of demyelination and subsequent remyelination in
this mouse model, that could be correlated with histochemical staining (37).

**Future research directions**

The current literature, as reviewed above, considering human post-mortem CNS tissue
specimens is limited, with most studies considering a nominal sample number as shown in
Table 1, which also summarises the studies completed in models of MS.

Studies focusing on animal models have shown spectroscopy to be a valuable tool in
probing the biochemical composition of samples otherwise deemed identical. The spectral
imaging of myelinating and remyelinating processes, for example, further demonstrated the
ability to differentiate between newly formed myelin and endogenous myelin, indicating the
remyelinating process generates myelin of a different composition. Nonetheless, studies
considering human samples are limited and concern only a small number of post-mortem
tissues as human CNS material is difficult to obtain. Furthermore, most studies focused on
the spectral distinction of MS lesions from control tissue of non-diseased subjects, which can
readily be achieved by macro and microscopic evaluation using luxol-fast blue (LFB) stain or
immunohistochemistry for myelin proteins to examine demyelination. As disease diagnosis
through tissue sampling is not feasible, the advantages of spectroscopy techniques such as
FTIR and Raman rely on their ability to reveal underlying biochemical changes not yet
detectable either macro or microscopically, for example on NAWM of MS cases, when
common techniques fail to recognize differences. Spectral data could potentially help to
understand the underpinning mechanisms of disease and advance research in the field by
probing deeper into the chemical composition of apparently normal areas of MS cases.

**FTIR and Raman spectroscopy analysis of post-mortem white matter MS tissue:**
**NAWM has a different signature**

Analysing four post-mortem brain samples obtained from UK MS Society Tissue Bank
(Imperial College London) we show FTIR signatures allow the distinction of normal control
WM from both active and chronic lesions, and more interestingly from the NAWM of MS
cases despite no visible demyelination being observed when staining NAWM with LFB. The
mean FTIR spectra of a brain tissue sample from control, NAWM, active lesion and a chronic
lesion are represented in Figure 1, where it is possible to observe that the symmetric and
anti- symmetric C-H stretches attributed to lipids ~2800-3000 cm\(^{-1}\) gradually decrease from
control to active lesion, as do the C-O and P-O stretches attributed to nucleic acids after 1000 cm\(^{-1}\).

This decrease in lipid content seems to be in line with the previous findings acknowledged in this review of published work and is in agreement with the well characterised process of demyelination which occurs in MS, providing support to the validity of this approach to the study of the biochemical composition of brain tissue in MS.

Principal Component Analysis (PCA) was further employed to highlight the variability existing in the recorded spectral data set. PCA of FTIR signatures allowed the distinction of normal control WM from both active and chronic lesions, as expected, but also differentiated NAWM of the MS cases from control white matter cases. 2-D PCA scatterplot is shown in Figure 2.

A clear distinction between all sample groups can be observed. If the distinction between control and chronic and active lesions were expected due to MS pathology, the separation between NAWM and control WM provides novel insights into the alterations in white matter in MS which might contribute to disease progression.

PCA was also employed to compare FTIR data from NAWM and control white matter samples and results are shown in Figure 3. Figure 3A indicates that NAWM and control white matter FTIR spectra separate according to the 1\(^{st}\) principal component (PC1) which accounts for 80.63% of the variation observed within the data set. The PC1 loading represented in Figure 3B shows that this separation is dominated by the negative loading of two main lipid assignments ~2800-3000 cm\(^{-1}\) indicating these are more intense in the control WM samples (in black on the negative part of the PCA plot (Figure 3A)).

These preliminary results demonstrate that FTIR spectroscopy can be applied to analysis of post-mortem WM tissue and successfully discriminate not only between lesion and control WM but also between NAWM and control, without requiring any additional techniques. Furthermore, they are suggestive of a significant decrease in lipid content in NAWM tissue in MS cases, which is not detected by current staining techniques or documented in the literature, but ought to be further investigated to better understand MS pathogenesis and the biochemical changes that lead to lesion formation. Finally, the spectral signatures of the fingerprint region also pointed to additional differences at the protein and nucleic acid level; these pose further questions as to which specific species (i.e. proteins) are being ‘lost’ in NAWM samples, which could contribute to the disease process.

Similarly, the samples were also analysed using a Horiba XploRA PLUS confocal Raman microscope, operating with 532nm laser light and 1800nm lines grating. Raman signatures
of the fingerprint region were analysed using PCA and results are shown in Figure 4. PCA score plots, in Figure 4A, showed the separation of NAWM (black) and control WM (green) only to be achieved on the third PC which account for approximately 2% of the variance found within the dataset. PC 1 and 3 loadings are shown in Figure 4B. Our group is currently investigating the Raman signatures of NAWM samples further and a full research paper will be published in due course.

FTIR and Raman spectroscopy analysis of biofluids

Much like tissues, biofluids exhibit vibrational spectra that have characteristic bands reflecting their bimolecular composition (4). There are several reports of the application of Raman and FTIR spectroscopy to the study of body fluids. Although blood and serum are most commonly used due to their easy, less-invasive availability other biofluids including cerebrospinal fluid (CSF), bile, urine, saliva, pancreatic juice, synovial and pleural fluids, which are considered to more closely reflect ongoing pathology in the associated diseased tissue, have also been studied. In Alzheimer’s disease, serum data from Raman spectroscopy allowed differentiation of Alzheimer’s patients from other dementia cases (38), whereas in a different study, plasma spectral data was used to grade mild, moderate and severe Alzheimer’s disease cases (39). FTIR spectroscopy showed Alzheimer’s patients’ plasma samples to be well delineated from normal ageing subjects (40) and the same was demonstrated for CSF (41). More recently, PCA-LDA allowed the distinction of the different types of mild, moderate and severe Alzheimer’s disease cases and controls, with 85% accuracy, when using white blood cells from patients, using FTIR spectra and about 77% when using the plasma spectra. These 83% accuracy values increased to 83 and 89% when only moderate and severe patient groups were being considered (42).

FTIR spectroscopy analysis of synovial fluid has been shown to allow differentiation of joints affected by rheumatoid arthritis, osteoarthritis, spondyloarthopathies and meniscal injuries(43); whereas a Raman study showed the ability to discriminate patients with low and high osteoarthritis severity (44). More recently, FTIR analysis of blood plasma for diagnosis of schizophrenia and bipolar disorders against a healthy control group has also been reported (45). A separation of all sample groups was observed using PCA, with assignments to lipids from lipoproteins, polypeptides, and phosphates associated to the DNA backbone being responsible for the separation; whilst PLS-DA allowed for the correct classification of all sample groups. Sensitivity and specificity results were highest when the full spectral range was considered, being respectively 100 and 100% for schizophrenia and 100 and 84.6% for bipolar disorder.
Overall, as demonstrated in this review and from our own FTIR preliminary data, spectroscopic techniques have the potential to advance our knowledge of MS pathogenesis. The analysis of post-mortem material, especially the comparison between NAWM and normal WM can provide insights into molecular changes unveiling novel disease mechanisms. And, although currently it cannot be applied for diagnostic purposes, due to the constraints of obtaining brain tissue specimens, other patient specimen samples such as CSF and blood might prove useful in the future to achieve a more rapid and accurate diagnosis and prognosis for people with MS, much like has been recently reported for other CNS diseases such as Alzheimer’s.

Conclusion

The understanding of the underlying mechanism that lead to disease pathology and specially disease progression is of great importance in neurodegenerative conditions, such as MS. Spectroscopy techniques have the ability to unbiased characterisation of the biochemical composition of post-mortem and clinical samples alike, thus able of providing insights into the underlying changes occurring in tissue and biofluids (i.e. blood and CSF) which in turn could be helpful to guide future in vitro research aimed at novel therapeutics.

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Table 1: Studies considered in this literature review. Sample specimen type and numbers included are indicated as well as the publication year and the spectroscopic technique used.

<table>
<thead>
<tr>
<th>Authors</th>
<th>Year</th>
<th>Tissue Sample species</th>
<th>No. samples</th>
<th>Spectroscopic technique</th>
</tr>
</thead>
<tbody>
<tr>
<td>Choo et al.</td>
<td>1993</td>
<td>Human</td>
<td>3</td>
<td>FTIR</td>
</tr>
<tr>
<td>LeVine et al.</td>
<td>1998</td>
<td>Human</td>
<td>10</td>
<td>FTIR</td>
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<tr>
<td>Fu et al.</td>
<td>2007</td>
<td>Mice</td>
<td>-</td>
<td>CARS</td>
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<td>2011</td>
<td>Guinea pigs</td>
<td>-</td>
<td>CARS</td>
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<td>2011</td>
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<td>Murine retinal organotypic cultures</td>
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Figure 1. Mean FTIR spectra for four post-mortem samples analysed in the preliminary study: chronic lesion (blue), active lesion (red), NAWM (green) and control (black). All samples were subjected to FTIR analysis at the Focas Research Institute, DIT, using a Perkin Elmer Spotlight 400N FTIR imaging system, incorporating a liquid nitrogen cooled mercury cadmium telluride 16x1, 6.25µm pixel array detector, and acquired by the Spectral Image software. FTIR images from the tissue sections (10µm sections) mounted on CaF$_2$ slides were recorded over the range 4000-800 cm$^{-1}$ in transmittance mode with a resolution of 4 cm$^{-1}$ and interferometer speed of 1.0 cm$^{-1}$/second at continuously varying magnification. The scans per pixel for background were 120 and, for images, 16 per pixel respectively. Spectroscopic data analysis was carried out in Matlab, version R2013 (Mathworks, CA, USA) according to protocols developed and routinely used in-house at DIT.

Figure 2. PCA of the four post-mortem samples FTIR data. (A) 2-D PCA scatterplot showing a separation between chronic (blue) and active lesion (red) and, NAWM (green) and control (black). (B) PC1 loading, responsible for the separation, is negatively dominated by peaks assigned to lipids around 2800-3000cm$^{-1}$.

Figure 3. PCA of the NAWM and control FTIR data. (A) The two dimensional PCA plot shows a separation between NAWM (black) and control (green) FTIR spectra in PC1 which explains 80.63% of the variation found in the data. (B) The PC1 loading is negatively dominated by peaks assigned to lipids around 2800-3000cm$^{-1}$.

Figure 4. PCA of the four post-mortem samples Raman spectroscopy data. (A) 2-D PCA scatterplots showing a separation between chronic (blue) and active lesion (red) and, NAWM (green) and control (black). (B) PC1 and PC3 loadings, responsible for the separation.
Figure 1

Absorbance (Arb. Units)

Wavenumbers (cm⁻¹)

Symmetric & Anti-symmetric C-H stretches (Lipids)

C-O and P-O stretches (Nucleic acids)

Chronic lesion

Active lesion

NAWM

Control
Figure 2

A

B
Figure 3

A

B
Figure 4

A

B

567