



Raman Analysis of Sperm Nuclear DNA Integrity

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Abstract

Raman Spectroscopy was evaluated as a non-invasive method of analysis of sperm DNA and the influence of UV irradiation on the sperm. The results show that Raman Spectroscopy, combined with multivariate analysis provide the reproducible and accurate information on DNA of sperm and the effect and location of damage.

Key words

DNA damage, sperm, human reproduction, Raman spectroscopy, Raman imaging, DuoScan

Introduction

Infertility has been and still it is a highly studied topic^{1,2,3}. It is known that sperm nuclear DNA (nDNA) integrity is a crucial factor for the success of male reproductive function. However, the status of sperm nDNA cannot be determined by the usual microscopic means which comprise routine semen analysis. Commercially available tests exist which assess the extent of sperm DNA fragmentation; however as they all result in the death of the sample they are of limited use to embryologists and ART (Assisted Reproductive Technology) clinics. The aim of our study, published in detail in "Human Reproduction"⁴, was to systematically appraise the utility of Raman microspectroscopy in analysing sperm nDNA status and can be compared to the results with established nDNA tests.

Figure 1a & 1b: Raman map and the characteristic Raman spectra of native sperm (the video image of the sperm in the inset). Three distinct regions in the sperm are observed: proximal head, distal head and tail, as well as small details as vacuoles.

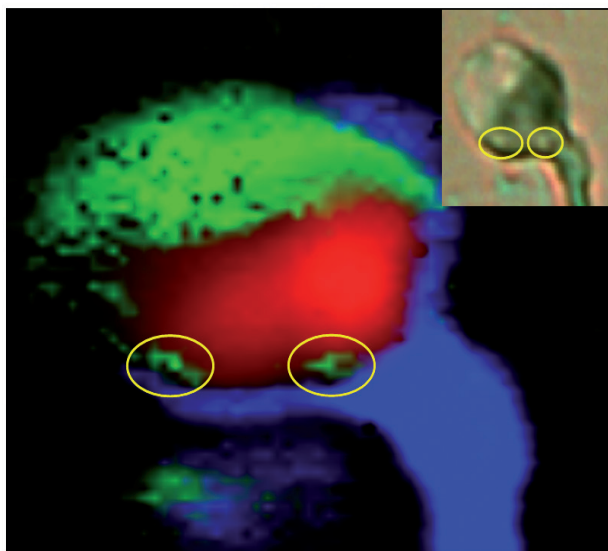


Figure 1a

Raman map on native sperm

The air-dried human sperm smeared on suprasil microscope slides were analysed using DuoScan™ imaging option to acquire $6.2 \times 6.5 \mu\text{m}^2$ Raman maps with a step of 100 nm. By assigning a specific colour to each of the chemical signatures, different regions of the sperm can be identified. The image was created using the Classical Least Squares fitting function of LabSpec software.

Three distinct chemical profiles were identified corresponding to the proximal head (containing DNA), the distal head (Acrosome) and the tail of the sperm (Flagellum). A sharp peak at 1000 cm^{-1} assigned to phenylalanine is seen only in the spectra from the tail and this, together with the sharp 1447 cm^{-1} methylene deformation peak indicates the presence of protein. The other two spectra contains a prominent 785 cm^{-1} peak associated with thymine, cytosine and the DNA backbone but only the distal head region contained a peak at 1092 cm^{-1} , indicative of the PO_4 backbone^{5,6}. The high spatial resolution of the Raman map allows the detection of small irregularities in the sperm head such as vacuoles (yellow circles).

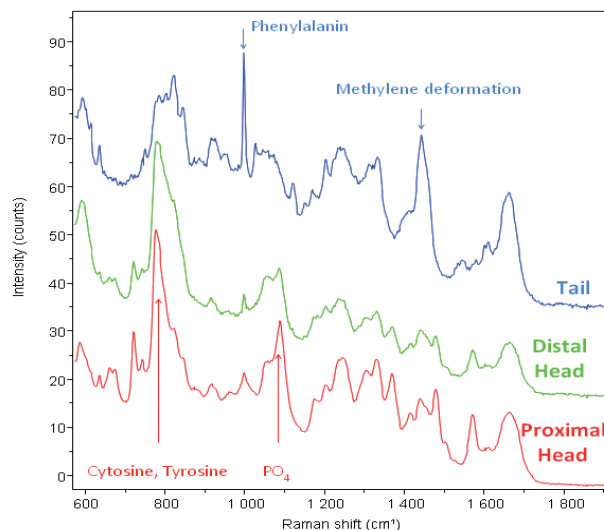


Figure 1b



Differentiation between native and UV irradiated sperm: chemometrics analysis

Raman measurements were carried out on native and UV irradiated (i.e. DNA damaged) sperm. Standard principal component analysis (PCA) was performed on three datasets (native sperm/observer W, native sperm/observer M and damaged sperm) to analyse a statistically meaningful number of measurements (200 sperm per sample). The results show that no difference is observed between the different observers' measurements on native sperm however they were clearly distinguishable from those obtained after UVB treatment (Figure 2).

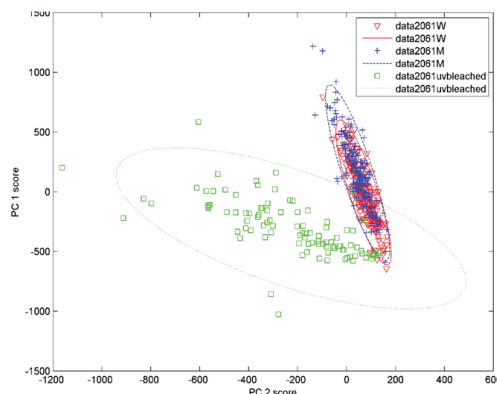


Figure 2: Principal component analysis: native sperm (red and blue, for two observers) and UVB-irradiated sperm (green).

In further analysis using the local spectral angle classification, the peak related to DNA PO_4 backbone showed a clear shift towards 1042 cm^{-1} and changes in its intensity after treatment (Figure 3). This difference indicates the dimerizations of nucleotide bases caused by UVB. Closer examination using wavelet decomposition confirmed the 1042 cm^{-1} shift and identified a possible second affected region ($1400\text{--}1600\text{ cm}^{-1}$) corresponding to protein–DNA interactions and thus susceptible to UVB damage.

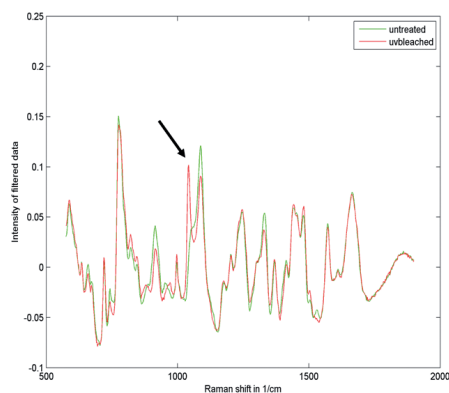


Figure 3: Averaged spectra showing the distinct shift at 1042 cm^{-1} (arrow) in spectra of irradiated (red) compared with that of the untreated human sperm (green).

The wavelet analysis of spectra of UV-irradiated samples allowed a visualization of the distribution of damaged and undamaged DNA. It clearly shows that the consequences of UVB irradiation are not homogenous throughout the sperm nucleus but are predominantly in the periphery, particularly under the acrosomal cap (Figure 4).

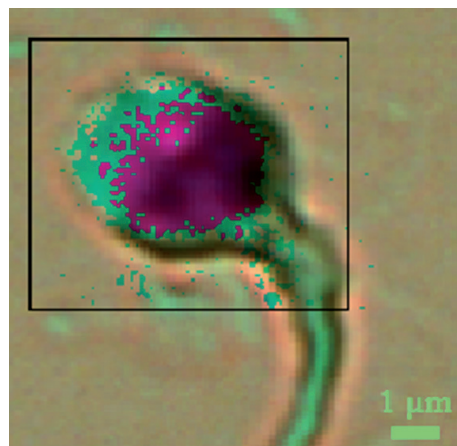


Figure 4: Overlay of human sperm showing the locations of spectra found to be indicative of intact (purple) and damaged (aqua) DNA.

Conclusions and perspectives

Once optimized, Raman microspectroscopy is a reproducible and reliable technique for sperm nDNA analysis with the acquisition of spectra being quick and easy. Even if the interpretation and analysis of the spectra can be complex, it can be overcome by the development of user friendly mathematical algorithms which would help to identify the presence of damaged DNA; and combined with mapping can give an indication of its distribution within the sperm head.

As no studies, ours included, have assessed living sperm, it is premature to advocate the use of Raman microspectroscopy for ART purposes. However if, as in the other cell types that have already been tested, the technique is found to provide a detailed chemical fingerprint whilst not damaging the integrity of living sperm then its application opens opportunities for basic and clinical andrology thus far beyond our technical capabilities. Studies could be undertaken on the nature, causes, impact and importance of sperm DNA fragmentation which in turn would lay the foundations for truly objective sperm selection.

Experimental parameters

A LabRAM Aramis Raman system was used in confocal mode with the HeNe laser (632.8 nm). The spectral resolution was 6.7 cm^{-1} . Peak assignments were based on the attributions in previous publications on Raman assessment of sperm^{5,6}.

References

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