Raman Spectroscopy: A Powerful and Non-Destructive Method to Label Stem Cells

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Introduction

- Raman Spectroscopy (RS) was discovered in 1928[1] and first used as a non-destructive method to characterise materials both chemically and structurally.
- The RS method involves the inelastic scattering of light, whereby a small difference in the incoming and outgoing light energy provides highly sensitive, spectroscopic information – a molecular-scale fingerprint of matter.
- In biological sciences, RS has been used to monitor changes in populations of *E. coli* bacteria[2], study cell cycle dynamics of osteosarcoma[3], assess quality of bone[4] and as a diagnostic tool for Alzheimer's disease and breast cancer[5].
- RS uniquely identifies biomolecules and provides a means of understanding stem cell differentiation processes[6].
- In this study, RS is used to identify multipotent stromal cells/mesenchymal stem cells (MSCs) isolated from various adult and foetal tissues that can be induced to differentiate into skeletal lineages including bone and cartilage[7].
- We demonstrate the sensitivity of RS as a means of non-destructively labeling MSC lines that are targeted for bone stem cell treatments, with a forward projection as to how RS can be employed to understand the process of stem cell differentiation for controlled tissue engineering and disease management.

Methods

- 4 cell lines of MSCs were prepared and then grown onto CaF_2 microscope slides.
- RS measurements were performed on single cells in each MSC line using a HORIBA XploRA micro-Raman instrument (Fig. 1) with 532 nm laser-light, x100 objective and 45s acquisition averaged over 2 repetitions.
- To ensure good statistics, a total of 100 spectra were taken from 20 cells in each cell line (5 spectra per cell). The spectra for each cell line were then averaged, baseline-corrected and fitted using the statistical software package IGOR Pro 6.32[8].
- Peak area ratios and peak intensity ratios were compared in the baseline-corrected spectra to determine possible Raman markers for identification of each of the 4 cell-lines.

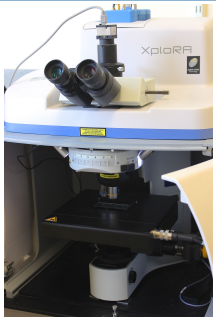
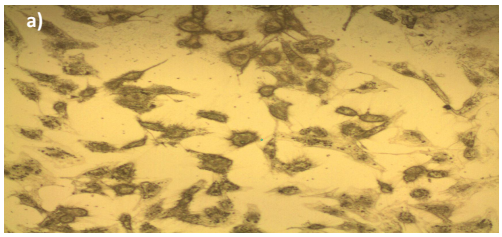
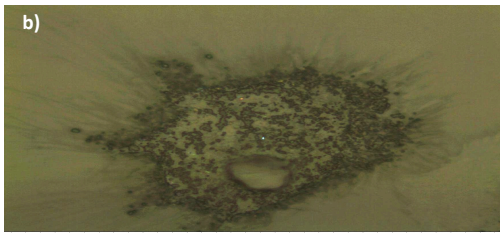


Fig.1: HORIBA micro-Raman XploRA consisting of a standard microscope with 3 laser wavelength options; 532 nm, 640 nm and 785 nm. x10, x50 and x100 objectives are used to collect Raman data and to perform white-light, optical microscopy. The latter is used to carefully monitor samples during analysis in order to prevent laser-induced damage.

Results

Fig. 2: (a) x50 and (b) x100 optical images of c101 MSCs. Five spectra were collected in different parts of each cell and 20 cells were randomly chosen in the slide. The lateral size of a single MSC is approximately 20 microns.





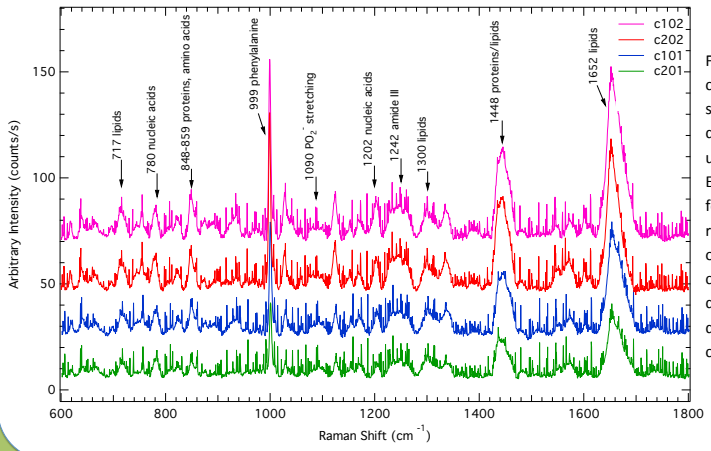


Fig. 3: Base-line corrected Raman spectra from the 4 different linages of undifferentiated MSCs. Each spectrum (shifted for comparison) represents the average of 100 spectra collected from 20 different cells in 5 different positions per cell.

Table 1: Peak intensity ratios relative to the 999 cm^{-1} phenylalanine peak. Raman markers, which are highlighted in yellow, are identified for ratios that lie outside the confidence band of the mean ± the std. deviation.

Peak Intensity Ratio	Cell Lines				Mean	Std. Deviation
	c101	c102	c201	c202		
999:1090	8.28	14.43	6.25	10.36	9.83	3.50
999:1448	2.07	2.28	1.84	1.89	2.02	0.20
999:1652	1.16	1.14	1.14	1.30	1.18	0.07
999:1202	5.03	4.78	5.86	5.53	5.30	0.49
999:717	5.41	6.22	4.76	6.18	5.64	0.70
999:847-858	3.85	4.03	3.91	4.57	4.09	0.33
999:780	4.52	6.01	3.53	5.19	4.81	1.05
999:1242	4.76	5.41	4.49	5.10	4.94	0.40
999:1300	4.43	6.20	3.36	5.51	4.88	1.25

Discussion and Conclusions

- Peak area and peak intensity ratios obtained from Raman spectroscopy analysis of 4 undifferentiated MSC lines were successfully used to determine markers for specific cell identification and molecular-scale fingerprinting, with peak intensity ratios being a more sensitive indicator for MSC identification.
- Our results demonstrate that Raman spectroscopy can be applied as a nondestructive method of determining MSC characteristics for monitoring and/or predicting cell differentiation potential.
- Future work will determine Raman markers from the parental cell line, as well as from differentiated MSCs. Comparison of these markers against those determined for the undifferentiated MSCs will provide a molecular-scale understanding of processes involved in MSC formation and differentiation. Such information will be used to develop targeted bone tissue engineering, thereby bringing MSCs to their maximal therapeutic potential.

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Acknowledgments

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