Characterisation of the differentiation of embryonic stem cells into defined neural lineages using vibrational microspectroscopy

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Stem cells have the potential of providing a source of specialised cells for future cell-based therapies. For example, transplantation of neural multipotent precursor (stem) cells (NPCs) is believed to be a possible therapeutic approach for repairing neurodegenerative disorders or traumatic damage of the central nervous system. A major hurdle in this research is the ability to identify and purify precursor cell lines derived from the stem cells, since they lack specific cell surface markers required for physical separation. Furthermore, conventional identification methods such as immuno-hisotchemical or fluorescence staining render the cells unviable making them unsuitable for clinical application. Accordingly, the development of label-free, noninvasive, spectroscopic methods is needed for cell identification and purification of stem cells in regenerative medicine.

A recently emerging approach to producing NPCs is through free-floating sphere-shaped aggregates of cells called embryoid bodies, which can be grown from suspended embryonic stem cells. We have compared mouse embryonic stem cells with neurospheres grown for one day in "chemically defined medium" to which growth factor FGF was added at 10 ng mL^{-1} to force cell differentiation along pathways leading to neural cell lines. There were very marked differences between spectra derived from ESCs and the one day old neurospheres, whereas spectra acquired from independent replicate ES cultures and neurospheres were similar. There were no gross morphological changes evident by light microscopy in the neurosphere-grown cells compared with the embryonic stem cells, however immunofluorescent staining revealed that differentiation of neural cell types had started, with positive identification of β-tubulin, nestin and GFAP.

Figure 1 shows the average second derivative FT-IR spectra from 3 replicate ES cell cultures and 3 replicate neurospheres after 1 day exposure to FGF. Marked changes are seen in bands associated with lipids (CH stretching bands at 2850, 2920 cm^{-1}; lipid ester carbonyl at 1735 cm^{-1}; and the asymmetric CH deformation band at 1450 cm^{-1}), proteins (amide I and II bands at 1645 and 1545 cm^{-1} respectively) and phosphorylated molecules (asymmetric and symmetric PO_2 stretches at 1250 and 1080 cm^{-1}). The ratio of the bands at 2920 and 2960 cm^{-1} increased in the neurosphere spectra indicating that the lipid increase involved largely unsaturated fatty acids, probably associated with membranes.

Following on from this initial study, we aim to compare spectra from homogeneous cell cultures with those from cells separated from developing neurospheres by fluorescence activated cell sorting (FACS), to track changes in differentiation in neurosphere cell populations over time.

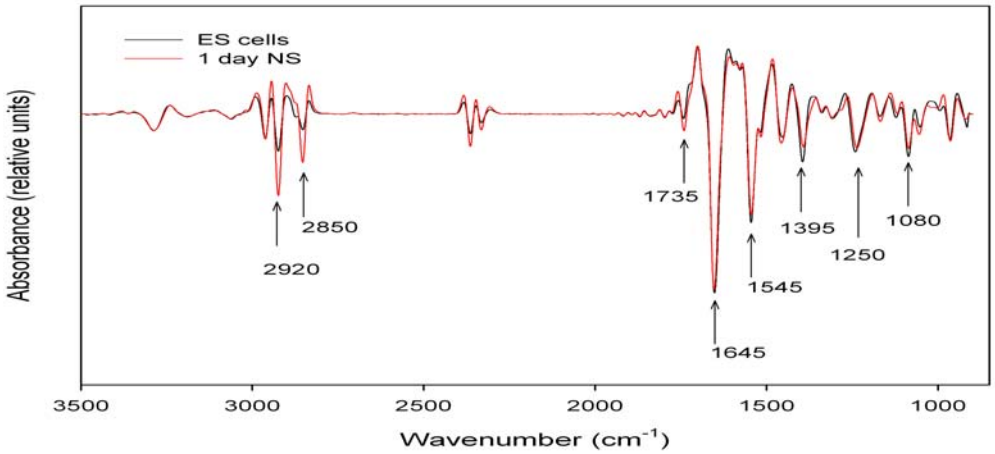

Figure 1 shows second derivative FT-IR spectra from a mouse embryonic stem cell line compared with FTIR spectra from neurospheres that have been undergoing differentiation for 24 h under the influence of the growth factor FGF.