Journal of Physical Chemistry & Biophysics

Kamma-Lorger et al. J Physic Chem Biophysic 2011, 1:1
DOI: 10.4172/2161-0398.1000103

Letter Open Access

FTIR has the Potential to Detect Stem Cells in the Bovine Corneal Stroma

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Abstract

Fourier Transform Infrared Spectroscopy (FTIR) was used for the first time to locate stem cells in the corneal stroma. An off-line microscope was used in Diamond light source and a bovine cryo-section was examined. Differences in spectra from two different regions suggest the existence of a cell population that has stem cell characteristics. Synchrotron FTIR would provide a greater scanning resolution than the off-line microscope used, therefore it could be an ideal technique to locate stromal stem cells in the cornea.

Keywords: Fourier Transform Infrared Spectroscopy ; Cornea; Stem cells; Stroma

Following corneal injury or refractive surgery, an intense wound healing response is initiated that seeks to restore tissue function and stability. Stem cell populations, thought to play a key role in corneal regeneration, are suspected to reside in both the epithelial and stromal layers. Corneal epithelial stem cells have been widely investigated in the past and are one of the most well understood stem cell systems in the body[1-3].However little is known about the stem cell population in the stromal layer (adjacent to the epithelium) which, following corneal trauma, is suspected to replace the normally quiescent stromal keratocytes of mesenchymal origin. Du et al. [4] proposed the existence of a stromal stem cell population in the cornea, but the exact location of these cells was not specified. In 2006 German et al. [2] successfully used FTIR spectroscopy to locate epithelial stem cells in the normal bovine cornea. Here we present the first documented application of FTIR techniques to detect stem cells in the corneal stroma.

An off line FTIR microscope in DIAMOND light source (Oxfordshire, UK) was used to obtain spectra from a healthy bovine cryo-section (30 μm thickness, limbus-to-limbus) at two different locations at 20 μm spatial resolution. (Settings used: 4 cm^{-1}, 128 scans, 15× objective, single channel detector). Statistical comparison (t-test) was employed in order to identify differences in the features of the FTIR spectra between the two different corneal areas. Comparison of the spectra between the two locations examined (Figure 1) revealed cell cycle differences previously attributed with stem cell characteristics [2]. These differences were statistically significant for spectral wavenumbers associated with C=O stretching vibration (i.e. ~1725 cm^{-1}), cell cycle changes (i.e. ~1315 cm^{-1}) and RNA expression (i.e. ~1110 cm^{-1}) [2,5]. These findings are related to cellular proliferative activity and according to German et al. [2] these properties describe stem cell characteristics and, in our case, point strongly to the possibility of another cell population, different to quiescent keratocytes, existing in the corneal stroma.

We hypothesise that key spectral features present in the data presented herein derive from a stromal stem cell population. However given the limited photon flux density of blackbody IR sources, combined with the extremely low absorbances typically obtained using corneal sections, these spectra may only be attributed in part to quiescent keratocytes. It is likely that a proportion of the signal is also due to other extracellular matrix components. Therefore, these preliminary findings require further investigation, ideally exploiting the higher light intensity available from a synchrotron source. This will render greater scanning resolution, facilitating collection of spectra even from single cells around 10 to 15 μm in diameter [2], and thereby giving a more detailed profile of the exact stromal cell population.

Acknowledgments

The authors would like to thank Dr. Che Connon for his useful commenting on the manuscript. This work was funded by a Medical Research Council 5-year programme grant (G0600755). Prof. Keith M. Meek is a Royal Society-Wolfson Research Merit Award Holder.

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Received November 07, 2011; **Accepted** December 26, 2011; **Published** December 29, 2011

Citation: Kamma-Lorger CS, Wehbe K, Boote C, Meek KM, Cinque G (2011) FTIR has the Potential to Detect Stem Cells in the Bovine Corneal Stroma. J Physic Chem Biophysic 1:103. doi:10.4172/2161-0398.1000103

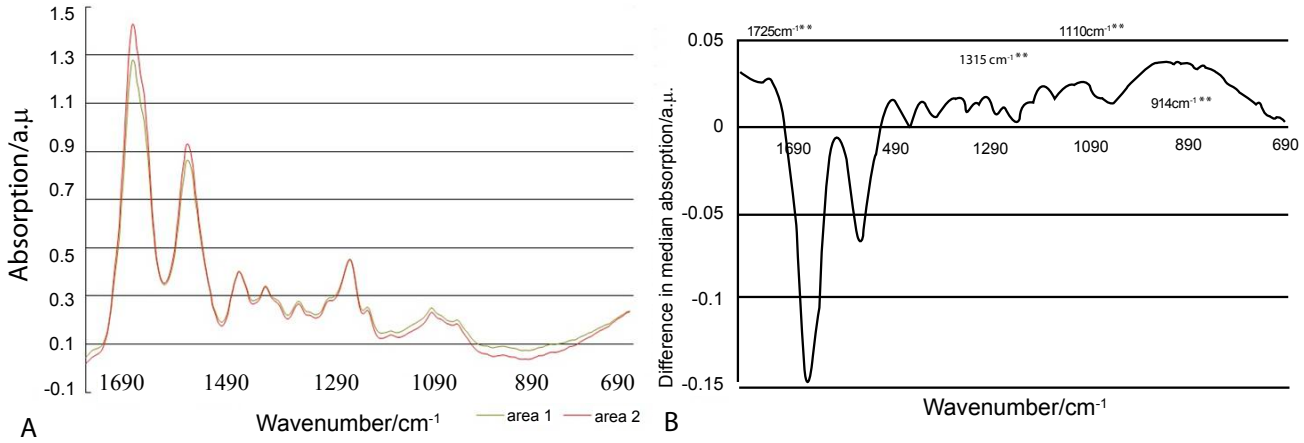

Figure 1: Comparison of FTIR spectra of two different locations in the corneal stroma (30 µm cryo-sections). Although it is widely known that the normal corneal stroma contains quiescent keratocytes, we found differences in spectra at the two different locations (A). Differences in spectra between the two examined regions (B). **Highly significantly different spectra, *Significantly different spectra; P<0.005.