Journal of Molecular Structure 933 (2009) 104–111

Contents lists available at ScienceDirect

Journal of Molecular Structure

journal homepage: www.elsevier.com/locate/molstruc

FTIR microspectroscopic imaging as a new tool to distinguish chemical composition of mouse blastocyst

Kanjana Thumanu [a,*], Waraporn Tanthanuch [a], Chanchao Lorthongpanich [b], Philip Heraud [c], Rangsun Parnpai [b]

[a] Synchrotron Light Research Institute (Public Organization), 111 University Avenue, Muang, Nakhon Ratchasima 30000, Thailand
[b] Embryo Technology and Stem Cell Research Center, School of Biotechnology, Institute of Agricultural Technology, Suranaree University of Technology, Nakhon Ratchasiam, Thailand
[c] Monash Immunology and Stem Cell Laboratories, and The Centre for Biospectroscopy, School of Chemistry, Monash University, Clayton, Victoria 3800, Australia

ARTICLE INFO

Article history:
Received 26 February 2009
Received in revised form 5 June 2009
Accepted 5 June 2009
Available online 9 June 2009

Keywords:
Focal plane array (FPA) imaging
Synchrotron-Infrared (SR-IR) mapping
Blastocyst
Principle Component Analysis (PCA)
Hierachical Cluster Analysis (HCA)

ABSTRACT

Synchrotron-Infrared (SR-IR) mapping and Focal plane array (FPA) imaging have been applied for discrimination of the three biochemical components of the mouse blastocyst. The mouse blastocyst consists of two clusters of cells known as the inner cell mass (ICM) formed within the blastocoel cavity and the thin layer of outer cells called the trophectoderm. Using Hierachical Cluster Analysis (HCA) and Principle Component Analysis (PCA), it can be shown that the composition and distribution of biochemical components within the blastocyst show differences in the protein secondary structure and the lipid content. It is worth noting that the secondary structure of the outer layer cells indicates more distinctive β-type secondary structure. The blastocoel cavity was observed to be predominantly α-helix. Significantly, the ICM region showed the predominant high absorption intensities of lipid content (CH_2, CH_3 symmetric and asymmetric stretching around 3000–2800 cm^{-1}). The results show agreement between both SR-IR mapping and FPA-IR imaging. We propose that the biochemical difference within the blastocyst, especially the high lipid content in the ICM region, could be involved in the process of lipid signaling during preimplantation. The use of both techniques is shown to be significant approach for revealing the biochemical components within the blastocyst.

1. Introduction

Traditionally, the gold standard for analysis of biological samples is based on histopathological assessment of the sample, related to a number of specific immunological staining procedures. The standard technique is used to determine cell types using specific antibody labels which recognize and bind specific antigens. Although, these methods are routinely used, they require accurate preparation, which are time-consuming and expensive. Fourier transform infrared (FTIR) microspectroscopy has been introduced as a new tool for chemical analysis of biological and biomedical samples [1–4]. This new technique is non-destructive and less expensive when compared with the immunostaining method for detection of specific proteins [5–7]. This alternative method is rapid as it allows the examination of the expression of different biomarkers simultaneously in a single absorption measurement. The FTIR spectrum of biological samples can provide detailed spectral information on cellular components such as nucleic acid, protein, lipid in the mid-IR spectral region between 4000 and 700 cm^{-1} known as "fingerprint region" of the spectral domain [8,9].

A more promising tool for a new generation of IR microspectroscopy is the Focal plane array detector (FPA) [2,10–14]. FTIR imaging based on FPA permits, rapid examination, of spatially resolved chemical information, from large sample areas, in a relatively short time, dramatically improving data acquisitions rate. Chemical imaging of the different structural components of biological samples can be imaged based on the vibrational signature of the sample components.

Synchrotron-Infrared (SR-IR) microspectroscopy has been introduced to identify molecular constituents in biological samples. With 100–1000 times brightness advantage of the SR source over the globar IR source, SR-IR microspectroscopy is well-suited for analyzing samples which require the characterization of a very small area to maximize the advantage of the SR-IR source [15–18]. The high brightness of the synchrotron source allows small regions to be detected with high signal to noise ratio and highly spatial resolution. However, SR-IR measurement has a main barrier as the fact that data can be acquired point by point spectral acquisition using synchrotron based mapping with a single point detector element. Therefore, the collection of individual spectra over large areas of sample is very time-consuming (takes hours).

During the process of embryogenesis by which the embryo is formed and develops, the fertilized eggs grow into a ball of cells

* Corresponding author. Tel.: +66 44217040; fax: +66 44217047.
E-mail address: kthumanu@slri.or.th (K. Thumanu).

0022-2860/$ - see front matter Crown Copyright © 2009 Published by Elsevier B.V. All rights reserved.
doi:10.1016/j.molstruc.2009.06.003

known as blastocyst. The blastocyst consists of the two primary cells; inner cell mass (ICM) and trophoblast (TE) cells. ICM are formed within the blastocoel (called blastocyst cavity) which is the fluid-filled center region of a blastocyst. The ICM develops into a fetus and a thin layer of outer cells called TE give rise to the placenta [19–21]. ICM is an important source of embryonic stem (ES) cells which could be induced and differentiated to form all cell types of the body. Moreover, the ES cells could also provide an important tool for medical examinations such as drug testing, embryo development and cell differentiation [22–24].

There are some literature reviews which attempt to understand the mechanisms of mouse embryo implantation [25–29]. It has been reported that mouse blastocyst releases a lipid anadanamide (AEA) that activates fatty acid amide hydrolase (FAAH) which is the metabolic gatekeeper for AEA signaling.

Normally, to understand the mechanisms responsible for embryo development and implantation events in mice, the standard techniques using molecular, biochemical and physiological approached were applied to study the expression of *N*-acylphosphatidylethanolamine-selective phosphate lipase D (NAPE-PLD), FAAH, CB1 receptor in the mouse oviduct and pre-implantation embryo [28,31].

Among the spectroscopic methods, FTIR is one of the vibrational spectroscopy techniques that can reveal the nature of a biological sample and can produce spectral maps of compositional and structural changes without marker on the molecular level. Recently, FTIR with FPA detectors have been used to identify the differentiation state of individual human mesenchymal stem cells with and without initiation of a differentiation process [35]. Also, conventional FTIR microspectroscopy has also been used to monitor spontaneous differentiation of murine ES cells [36]. In this study, we apply both techniques to distinguish structural information by creating a spectroscopic database which contains statistical information from FTIR spectra of the mouse blastocyst. This work helps us to reveal different biochemical components of mouse blastocyst especially in the ICM region which could be involved in the regulation of the cell signal during the process of blastocyst activation. Coupled with PCA and HCA, we demonstrated that FPA-IR imaging and SR-IR mapping enabled us to identify spectral changes and distinguish three biochemical components of the mouse blastocyst.

2. Materials and methods

2.1. Animals

Female outbred and inbred stock mice (ICR and C57BL/6 strain; 4–6 weeks old) were superovulated with 10 IU pregnant mare serum gonadotropin (Sigma). This was followed by 10 IU human chorionic gonadotropin (hCG, Sigma; i.p.) 48 h later, and then by natural mating with the same strain of male mice. Two-cell (2C) embryos were collected at 43–45 h after the hCG injection from the oviducts and then cultured in 20 ml drops of CZB media [37] under mineral oil with 5% CO_2 in air at 37 °C until development to the blastocyst.

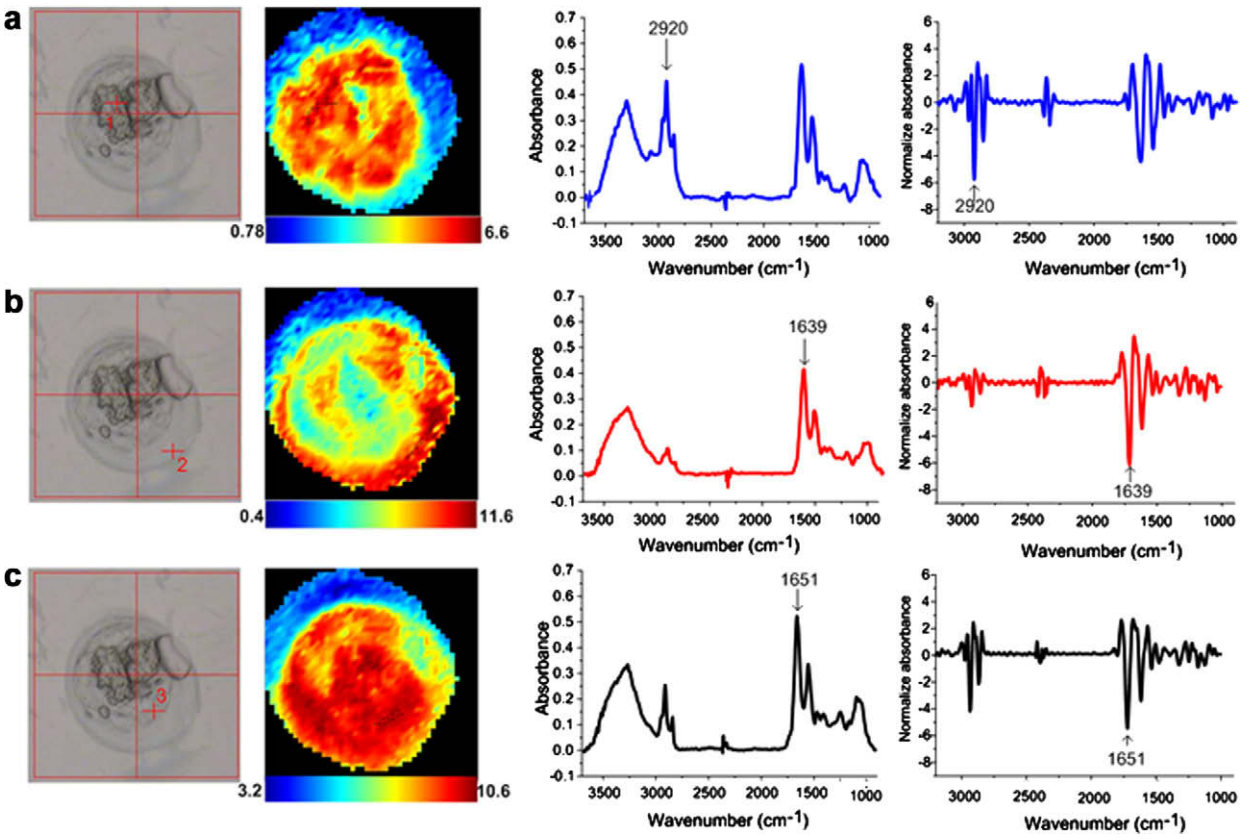

Fig. 1. Molecular functional group image of the mouse blastocyst analyzed by FPA-IR Imaging. A mosaic of 4 FTIR images was sequentially recoded in a 2 × 2 pattern which covered an area of 340 × 340 μm. The IR image (64 × 64 pixels) was generated with a 32 × 32 pixel FPA detector (128 scans, 6 cm^{-1} resolution). The color-coded has been used to indicate absorbance, with the highest absorbance (red color) and the lowest absorbance (blue color). Visual image and FTIR Overview image based on functional groups map obtained from original spectra and 2nd derivative and normalized at absorbance value (a) 2920 cm^{-1}, (b) 1635 $cm^{-1}$and (c) 1653 cm^{-1}. Representative spectra corresponding to the pixel at the cross-hair in the visible image at position 1–3. (For interpretation of color mentioned in this figure, the reader is referred to the web version of this article.)