CHAPTER I
INTRODUCTION

1.1 Introduction

Pluripotent stem cells (PSCs), derived from the epiblast of mammalian blastocysts, have the dual capacity for unlimited self–renewal and differentiation into derivatives of all three germ layers (Evans and Kaufman, 1981). In rodents, PSCs can exist in two distinct states: the naïve state, represented by embryonic stem cells (ESCs) derived from inner cell mass (ICM) of the blastocysts, and the primed state, represented by epiblast stem cells (EpiSCs) derived from post implantation epiblast tissue (Nichols and Smith, 2009). These states are also recognized in human and non–human primates (NHPs), including rhesus macaques (*Macaca mulatta*), where PSCs can stabilize in either a naïve and primed configuration depending on culture conditions and signaling inputs (Anwised et al., 2023). Naïve and primed PSCs differ markedly in their transcriptional, epigenetic, and metabolic profiles, which in turn have a profound impact on their functional properties (Weinberger et al., 2016). Notably, only naïve PSCs in the naïve state exhibit the capacity to integrate into host preimplantation embryos and contribute to development, as demonstrated in rodents (Nichols and Smith, 2009), rabbits (Pham et al., 2025), and cynomolgus monkeys (Li et al., 2023). These functional differences are underpinned by distinct regulatory networks and chromatin landscapes: naïve PSCs display global DNA hypomethylation, two active X chromosomes in female cells, and reduced levels of H3K27me3 at developmental gene loci, whereas primed PSCs show higher DNA methylation, one inactive X chromosome in female cells, and accumulation of repressive histone marks (Weinberger et al., 2016). Identifying the pluripotency state of a given PSC line traditionally requires multimodal analyses, including bulk RNA sequencing (Wang et al., 2009), immunostaining (Cregger et al., 2006), state–specific markers (e.g., KLF4, TFCP2L1 for naïve; OTX2 for primed), quantitative PCR (qPCR) (Higuchi et al., 1993; Bustin, 2000), reporter–based functional assays, and epigenomic profiling. These approaches, while informative, are often labor intensive, sample preparation, time consuming procedures, a large number of samples needed, not readily applicable for routine quality control, and in addition, each sample preparation method can available for only one marker at a time. Although qPCR is an accurate method to verify such specific markers, a large number of cells are required for such testing. Moreover, the presence of transcripts

does not guarantee expression at the protein level (Chonanant et al., 2011). In this context, Fourier–transform infrared (FTIR) spectroscopy offers an attractive, non–invasive alternative for cell characterization (Cao et al., 2013b).

FTIR microspectroscopy analyzes vibrational energy absorption in molecular bonds, providing a composite biochemical fingerprint of cells. The technique enables rapid, can measure both transmission and reflection (Thumanu et al., 2011) and label–free quantification of major cellular components—including proteins, lipids, carbohydrates, and nucleic acids—at single cell resolution. Importantly, changes in cell state are accompanied by alterations in molecular composition and structure, which can be detected in specific IR absorption bands. For example, shifts in the amide I and II regions (1700–1500 cm^{-1}) reflect differences in protein secondary structure and abundance; CH_2 and CH_3 stretching modes (3000–2800 cm^{-1}) are indicative of membrane lipid composition and fluidity; and bands in the 1200–800 cm^{-1} region reflect changes in nucleic acid conformation and RNA/DNA content (Ami et al., 2008; Bassan et al., 2009; 1Thumanu et al., 2011; Cao et al., 2013b). Nucleic acid absorption region (1050-850 cm^{-1}) pointed towards high levels of messenger RNA (mRNA) translation and production of specific proteins indicating that the cells harbor a new phenotype. Furthermore, the DNA/RNA hybrid bands at 954 cm^{-1} and 899 cm^{-1} were also observed, suggesting transcriptional switch that started with the differentiation of the cells (Ami et al., 2008). Principal component analysis (PCA) and subsequent linear discriminant analysis (LDA) were employed to effectively segregate stem cell spectra into distinct clusters, facilitating the identification of the most noteworthy spectral changes.

The first study that aimed to characterizing specific ESCs derived cells (Ami et al., 2008). The authors used the FTIR method to monitor the spontaneous differentiation of mouse embryonic stem cells (mESCs). Heraud et al. (2010) utilized focal plane array (FPA) FTIR microspectroscopy to examine human embryonic stem cells (hESCs). Their findings suggest that FTIR spectroscopy possesses the capability to characterize the macromolecular composition of cells and effectively discriminate hESCs that differentiate into either ectoderm, endoderm, or mesoderm. This discriminatory potential is further supported by multivariate data analysis techniques such as PCA, partial least squares discriminant analysis (PLS–DA), and artificial neural networks (ANN). Previous bio–spectroscopic studies have successfully applied FTIR to distinguish undifferentiated from differentiated cells, to assess stem cell lineage commitment, and to monitor reprogramming efficiency (Cao et al., 2013a). However, no study has explored its application for distinguishing between naïve and primed states of pluripotency, particularly in non–human primates. Establishing such a methodology

could provide a powerful tool for monitoring cell identity and quality in basic and translational stem cell research. There are 2 types of infrared radiation detectors; 1) mercuric cadmium telluride (MCT) detector measures 1 sample/ 1 spectrum and 2) FPA detectors measure maximum 4,096 spectrums in one time; FPA detectors can create a distribution diagram faster than MCT detectors (Wold et al., 1987). A technique known as FPA infrared imaging with multichannel detectors has recently become available for analyzing cells and tissues with allowing to measure all data points from each detector element simultaneously.

In this study, we applied FPA–FTIR microspectroscopy to rhesus macaque embryonic stem cells (rhESCs) cultured under two distinct conditions: the well–established FGF2/KOSR medium, which supports the primed state, and a novel medium we developed, ALGöX (Pham et al., 2025), designed to support a naïve-like pluripotent state. ALGöX consists of fibroblast conditioned N2B27 medium supplemented with activin A, leukemia inhibitory factor (LIF), PKC inhibitor Gö6983, and tankyrase inhibitor XAV939. This combination aims to inhibit MEK/ERK and WNT signaling while maintaining key self–renewal cues, based on pathways shown to stabilize naïve pluripotency in rodent and human systems. We show that rhESCs cultured in ALGöX exhibit distinct spectral features compared to those in FGF2/KOSR.

1.2 Research objectives

1) To characterize and compare between passage cells in primed and naïve-like states rhESCs by standard methods.
2) To characterize and compare between passage cells in primed and naïve-like states rhESCs by FPA–FTIR microspectroscopy.
3) To correlation analysis between standard characterization methods and FPA–FTIR microspectroscopy.
4) To comparison chemical change from each passage on the biological replication by FPA–FTIR microspectroscopy.

1.3 Research hypotheses

1) rhESCs had different chemical compositions those could characterize and identify primed and naïve-like states.
2) FTIR microspectroscopy could distinguished primed and naïve-like states rhESCs.

1.4 Scope of research

1) rhESCs-FGF2/KOSR cells (primed state) and rhESCs-ALGöX cells (naïve-like state) used in this study were obtained from INSERM, Stem Cell and Brain Research Institute, Lyon, France. Both types of cells were characterized by immunocytochemistry (ICC), RNA sequencing (RNA–seq) and FPA–FTIR microspectroscopy techniques.
2) FTIR spectra of rhESCs in primed and naïve-like states of pluripotency were analyzed used FPA–FTIR microspectroscopy.