CHAPTER V
CONCLUSION

This work represents the first application of FTIR microspectroscopy to characterize and distinguish primed from naïve-like rhESCs. The FTIR–based findings were validated against two established methods immunocytochemistry and RNA sequenching which confirmed that naïve-like cells possess higher protein content, while primed cells are enriched in lipids. The three analytical approaches were complementary: immunocytochemistry localized proteins, RNA sequencing provided gene–level resolution, and FTIR offered a rapid, cost–effective overview of cellular biochemical composition.

Immunolabeling confirmed that rhESCs in both culture conditions expressed core pluripotency markers; OCT4, NANOG, and SOX2. However, the expression of these markers was more stable in rhESC-ALGöX cells across passages, while expression in rhESC-FGF2/KOSR peaked at passage 60 and declined thereafter. Critically, a key distinction was the exclusive detection of the primed–state marker OTX2 in rhESC-FGF2/KOSR cells. Conversely, rhESC-ALGöX cells showed robust expression of naïve markers, including KLF17, ALPPL2, TFCP2L1, and TFAP2C, confirming a naïve pluripotency signature. These findings were further validated by RNA sequencing, which demonstrated a clear transcriptional divergence between the two cell populations. The rhESC-FGF2/KOSR cells exhibited elevated expression of primed–state genes (*NODAL*, *OTX2*, *ETV4*, *BMP4*, *FST*, and *SOX3*). In contrast, rhESC-ALGöX cells showed high expression of naïve–associated genes (*KLF2*, *DPPA2*, *DPPA3*, *ZFP42*, *PRDM14*, and *TFCP2L1*). These results collectively indicate that the ALGöX medium successfully reprogrammed primed rhESCs to a stable, naïve-like pluripotent state.

FTIR microspectroscopy served as a robust, label–free method for distinguishing between the two pluripotent states. Analysis of the second–derivative spectra revealed distinct biochemical profiles. Naïve-like rhESCs exhibited significantly higher integrated absorbance in the amide I and amide II regions, indicating higher protein synthesis. They also showed strong nucleic acid peaks at 1239 and 1085 cm^{-1}, suggesting increased transcriptional activity. In contrast, primed rhESCs showed stronger lipid–associated absorbance, particularly in the CH_2/CH_3 stretching region (2921–2852 cm^{-1}) and the ester carbonyl stretch at 1741 cm^{-1}, consistent with a metabolic shift towards increased lipid utilization.

PCA effectively segregated the primed and naïve-like cells into two distinct clusters based on their spectral signatures, with PC–1 accounting for 64% of the variance. Additionally, a PLS–DA model was developed, which achieved 100% specificity and 100% sensitivity in classifying the two cell states. This demonstrates the high predictive power of FTIR microspectroscopy for identifying rhESCs. These FTIR–based biochemical distinctions were consistent with the results from immunocytochemistry and transcriptomic data, establishing FTIR microspectroscopy as a powerful new approach for monitoring stem cell identity and quality.