I. INTRODUCTION

1.1. Background

Human adipose-derived stem cells (hADSC) are mesenchymal stem cells that are able to differentiate into several lines of cells, such as osteoblasts (bone cells), chondrocytes (cartilage cells), myocytes (muscle cells), and adipocytes (fat cells) (Cui et al., 2024). These stem cells could be found within adipose tissues, and are retrieved using minimally-invasive methods such as liposuction, making them a promising candidate for its use in regenerative medicine, such as cartilage formation and osteoarthritis research (Biniazan et al., 2024). A key aspect of hADSC identity lies in their characteristic morphology and stemness profile, which distinguish them from more terminally differentiated cell types. Undifferentiated hADSCs typically exhibit a fibroblast-like, spindle-shaped morphology, whereas chondrocytes adopt a rounder, spheroidal, more compact form consistent with their specialized function in cartilage matrix production (Al-Maslamani et al., 2022). Assessing these morphological traits is therefore a critical step to ensure cell purity, confirm stem cell identity, and detect early phenotypic drift or spontaneous differentiation, which are factors that can significantly influence the cell and its use in downstream applications.

Traditional confirmation of stemness relies on molecular assays such as qPCR and Western blot to evaluate markers like CD90, CD105, and transcription factors associated with multipotency (Wang et al., 2022; Yu & Li, 2022). However, these techniques are time-consuming, require specialized reagents, and may be impractical in settings where only imaging data are available. In contrast, quantitative morphology-based assessment offers a rapid, non-destructive way to evaluate stem cell characteristics directly from bright-field images. Quantitative morphometric analysis offers an objective alternative to subjective manual microscopy by extracting measurable features such as area, eccentricity, axis lengths, solidity, and perimeter (Kenry, 2024; Vadori et al., 2025). These morphological descriptors are highly informative because transitions in cell shape often accompany early lineage commitment or loss of multipotency (Geng & Kidder, 2025). As such, computational phenotyping provides meaningful insight into hADSC behavior, especially when assessing subtle differences between experimental hADSC populations, baseline controls, and chondrocytes.

CellProfiler, an open-source image analysis software, is widely used for high-content single-cell quantification, particularly in fluorescence microscopy where high-contrast staining enables robust segmentation (Schüssele et al., 2022). However, its application to bright-field microscopy of primary stem cells remains relatively uncommon, as the low contrast of bright-field images makes automated segmentation more challenging. Utilizing CellProfiler to extract single-cell morphological features from bright-field images of hADSCs therefore represents a novel approach. By

enabling scalable, quantitative analysis of structural characteristics without fluorescent labeling, this method provides a powerful way to assess stem cell morphology and compare hADSCs against differentiated chondrocytes with greater precision and reproducibility.

1.2. Aim of Study

This study aims to quantitatively characterize the morphology of human adipose-derived stem cells (hADSCs) obtained from bright-field microscopy images and to compare these morphological signatures against control hADSCs and differentiated chondrocytes to evaluate hADSC quality and may further reinforce their stemness status and stem cell-like features.

1.3. Hypothesis

It is hypothesized that the cultured human adipose-derived stem cells (hADSC) *in vitro* expressed a distinctive elongated, spindle-like, fibroblast-like morphology under the bright-field inverted microscope. The morphology is also expected to be different between hADSC as well as chondrocytes as one of their main committed cell lineages, with chondrocytes expressing a more spheroidal morphology.