Impact of isolation method on cellular senescence and quality of adipose-derived stem cells from micro-fragmented adipose tissue for musculoskeletal regeneration

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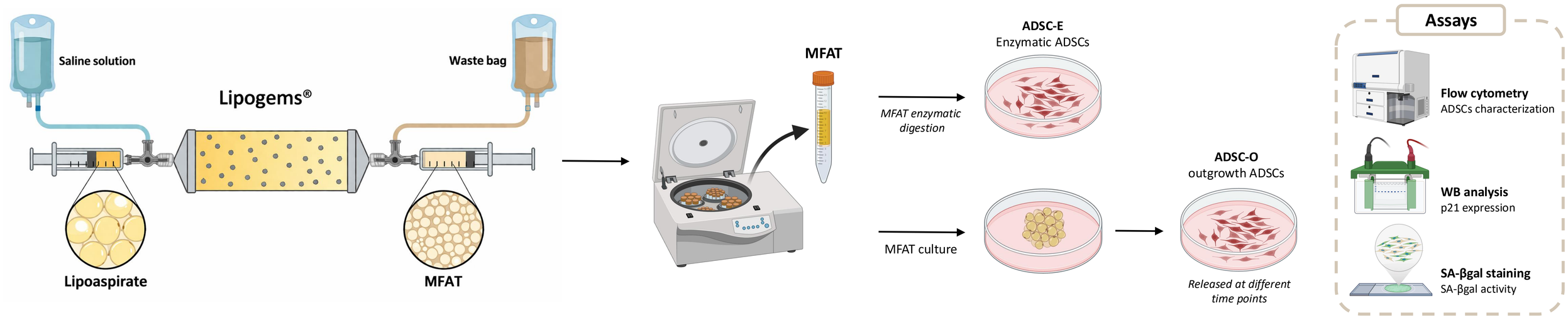
Background

Despite the promising outcomes of stem cell-based therapies, clinical failure rates remain significant. One of the main contributing factors is cellular senescence, which frequently arises during the in vitro expansion phase required for large-scale cell production. Senescent mesenchymal stem cells (MSCs) exhibit impaired proliferative capacity, altered secretory profiles, and reduced differentiation potential, ultimately compromising their therapeutic efficacy and regenerative performance.

Aim

In this study, we compared the senescence profile of adipose-derived stem cells (ADSCs), isolated from enzymatically digested micro-fragmented fat (MFAT) obtained with Lipogems technique, with those obtained via spontaneous outgrowth from cultured MFAT. By analyzing differences in senescence-associated markers between these two isolation strategies, our work aims to elucidate if the preservation of the native stromal vascular niche reduces cellular aging, contributing to optimize adipose-derived stem cell preparation and improving the overall efficacy of MSC-based regenerative therapies.

Workflow



Results

Figure 1

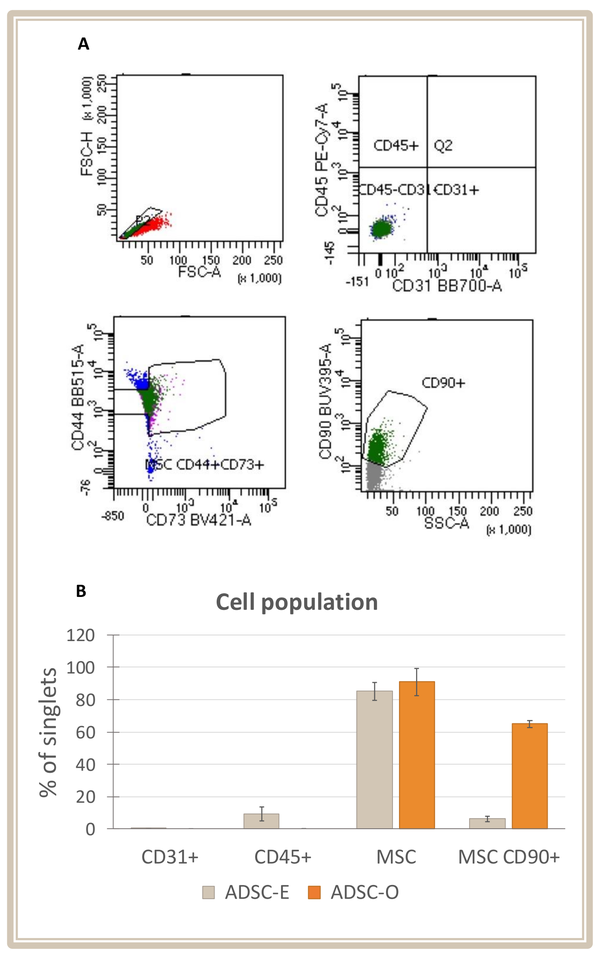


Figure 1 - Flow cytometry analysis

Both isolation methods yielded more than 80% MSCs ($CD44^+CD73^+CD45^-CD31^-$), with a significantly higher percentage observed in cells obtained without enzymatic digestion. Moreover, a higher percentage of $CD45^+$ cells was observed in enzymatically isolated ADSCs (ADSC-E), whereas a higher percentage of $CD90^+$ cells was found in ADSCs obtained without enzyme digestion (ADSC-O).

Figure 2

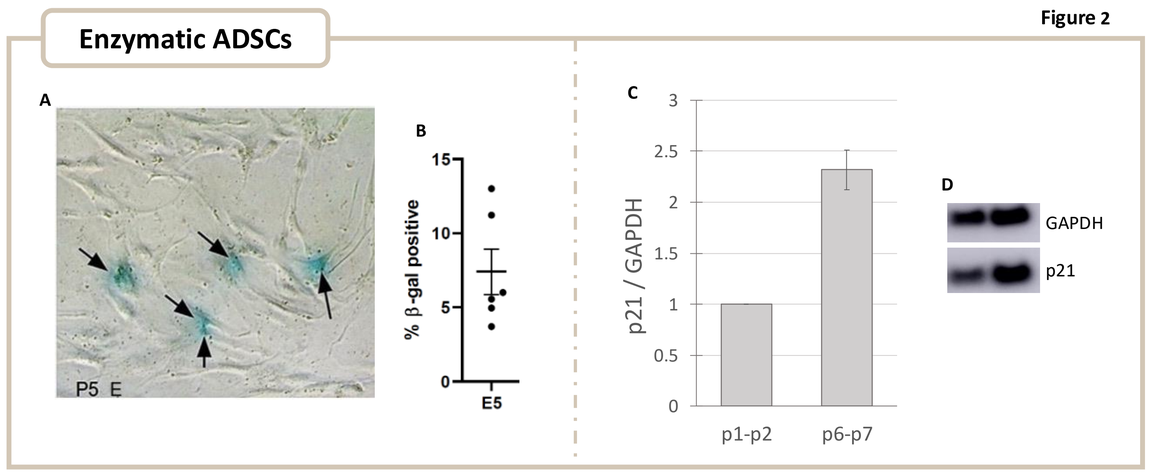


Figure 3

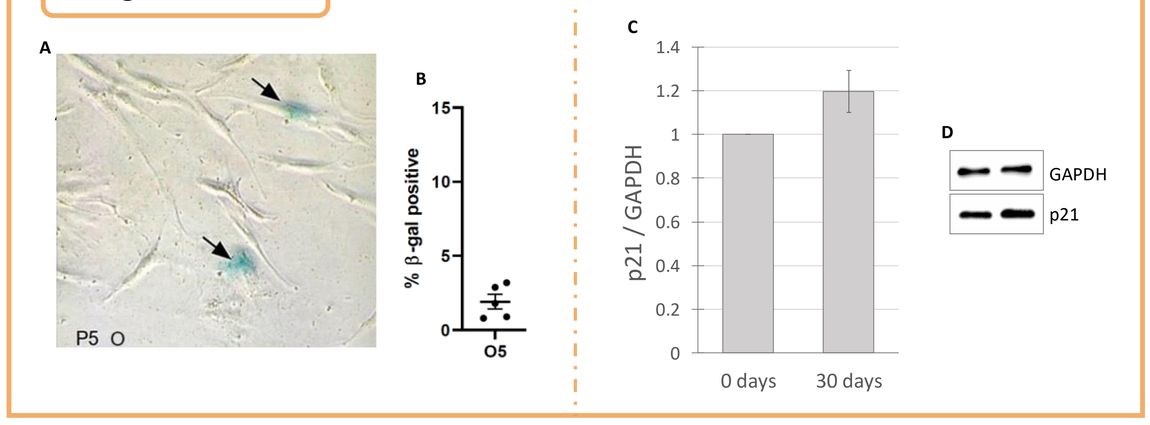


Figure 2, 3 - senescence evaluation

ADSCs isolated by enzymatic digestion (ADSC-E) exhibited premature senescence during expansion, as demonstrated by increased p21 expression and SA-βgal activity. Notably, p21 expression significantly increased over passages, indicating enhanced cellular senescence and reduced phenotypic stability.

In contrast, ADSC-O maintained a more stable phenotype, with relatively stable p21 levels and low SA-β-gal activity, showing only a modest increase over time.

Overall, these findings suggest that enzymatic isolation negatively affects both phenotypic stability and senescence, whereas preservation of tissue structure during outgrowth exerts a protective effect that helps preserve the regenerative potential of ADSCs.

Conclusion

Both isolation methods generated ADSCs with high MSC yields. However, the outgrowth approach resulted in a higher MSC percentage with lower $CD45^+$ contamination and higher $CD90^+$ expression, indicating improved stemness. Notably, this method enabled a sustained release of ADSCs from the tissue over multiple time points with cells maintaining relatively stable p21 levels over time. In contrast, ADSC-E showed premature senescence, with increased levels of p21 and SA-βgal during expansion, indicative of enhanced cellular senescence. These findings suggest that the native adipose tissue structure and microenvironment supports the maintenance of ADSCs viability and stemness *in vitro* and exert a protective effect that preserves regenerative potential compared to traditional enzymatic method.