Microfragmentation for processing stem cells from adipose tissue is promising when compared to enzymatic digestion for the treatment of osteoarthritis

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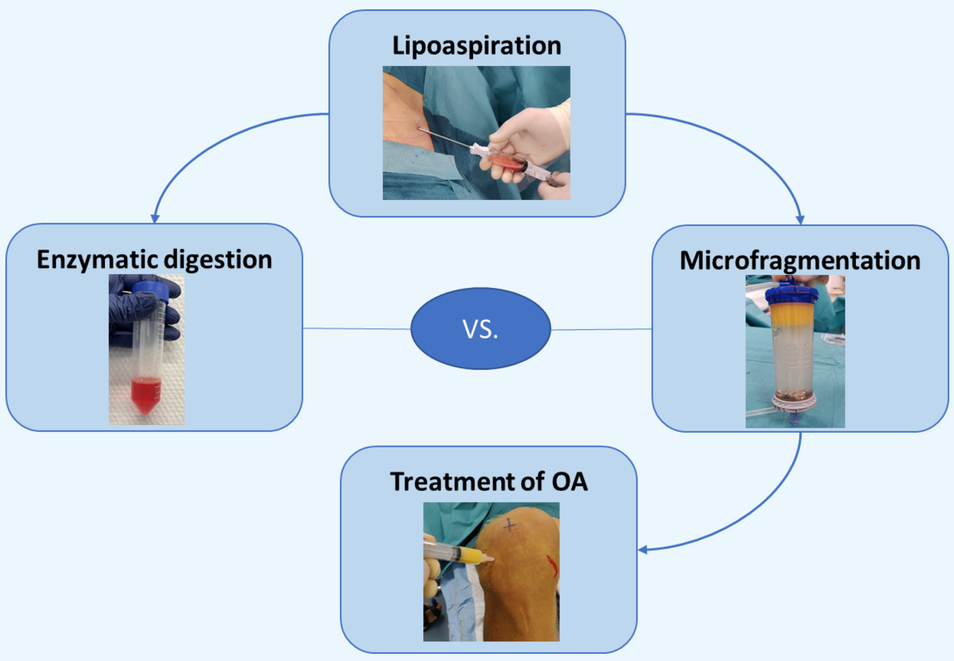
INTRODUCTION

Intraarticular treatment of knee osteoarthritis with adipose tissue-derived stem cells has shown promising results[A,B]. Until now, standard processing of lipoaspirates for therapeutic use consists of enzymatic digestion and cell expansion prior to injection. However, complex regulatory issues related to application of enzymatically treated and expanded cells have led to development of mechanical microfragmentation to harvest stem cells ready for treatment[C].

OBJECTIVE

To assess quantity, viability, and cell type of stem cells from abdominal adipose tissue when processed with microfragmentation compared to enzymatic digestion.



MATERIALS AND METHODS

Abdominal adipose tissue from knee osteoarthritis patients was processed with microfragmentation and enzymatic digestion, respectively. Cell type, quantity, and viability was investigated using trypan blue staining and flow cytometry. Statistical analysis was performed using paired t-tests. p-values <0.05 were considered statistically significant.

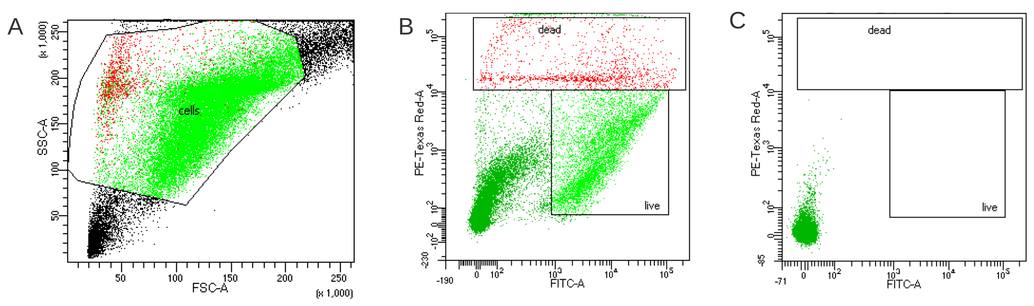


Figure 1: *Flow cytometry gating strategy for Live/Dead Assay to determine cellular viability of stem cells.* ***(A)*** *Cells were selected based on Forward scatter (FSC) vs. Side scatter (SSC) using verified adipose tissue-derived stem cells as a positive control and peripheral blood cells as a negative control.* ***(B)*** *Gating on live and dead cells of stained sample from microfragmented adipose tissue.* ***(C)*** *Unstained control.*

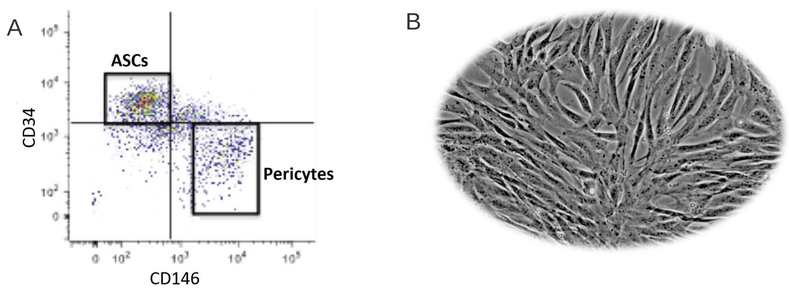


Figure 2: (A) *Flow cytometric gating on adventitial stem cells (ASCs) and pericytes from microfragmented adipose tissue based on CD34 and CD146 surface marker expression.* ***(B)*** *Cultured adipose tissue-derived stem cells used as size control for flow cytometry.*

TAKE HOME MESSAGES

- Microfragmentation is promising to harvest clinically relevant stem cells from adipose tissue with high cellular viability
- Enzymatic digestion provided more nucleated cells than microfragmentation, but the stem cell content per total nucleated cell count was higher when using microfragmentation
- Several clinically relevant stem cell types were identified in abdominal adipose tissue following microfragmentation

RESULTS

15 patients, age (mean (SD) 50.9 (8.5)).

Trypan blue staining

- Enzymatic digestion provided more nucleated cells $3.9x10^6$/mL ($2.5x10^6$/mL) than microfragmentation $1.0x10^6$/mL ($0.5x10^6$/mL), p<0.01
- Enzymatic digestion gave higher total nucleated cell viability 90% (4%) compared to microfragmentation 80% (6%), p<0.01

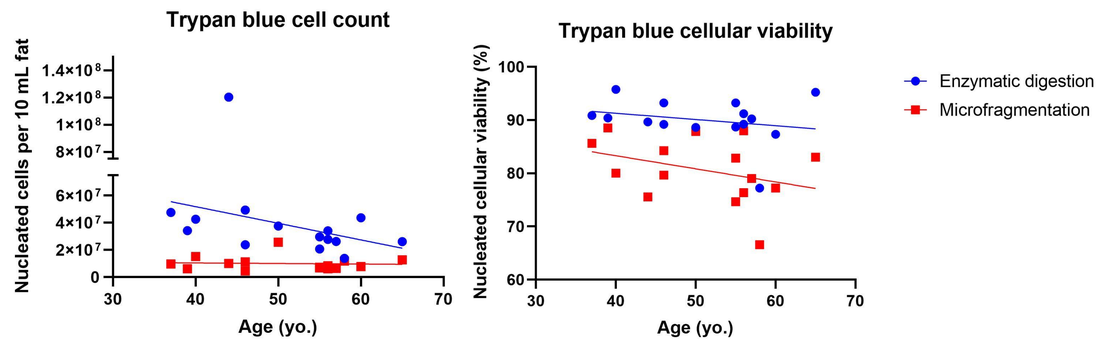


Figure 3: *Trypan blue staining results on yield and viability (%) of total nucleated cells per 10 mL adipose tissue when processed with enzymatic digestion or microfragmentation.*

Flow cytometry – gating on stem cells

- Equally high **viability** was identified for enzymatic digestion 82% (4%) and microfragmentation **84%** (3%), p=0.17
- Higher **stem cell content** per total nucleated cell count for microfragmentation **10%** (2%) compared to enzymatic digestion 6% (2%), p<0.01
- Stem cell types identified in microfragmented adipose tissue:
  - Adventitial stem cells (ASCs) ($CD31^-/CD45^-/CD34^+/CD146^-$)
  - **Pericytes** ($CD31^-/CD45^-/CD34^-/CD146^+$)
  - Mesenchymal stem cells ($CD34^-/CD45^-/CD146^-/CD90^+/CD105^+$)
  - **$CD271^+$ stem cells** ($CD31^-$/ $CD45^-/CD90^+$ /$CD271^+$)

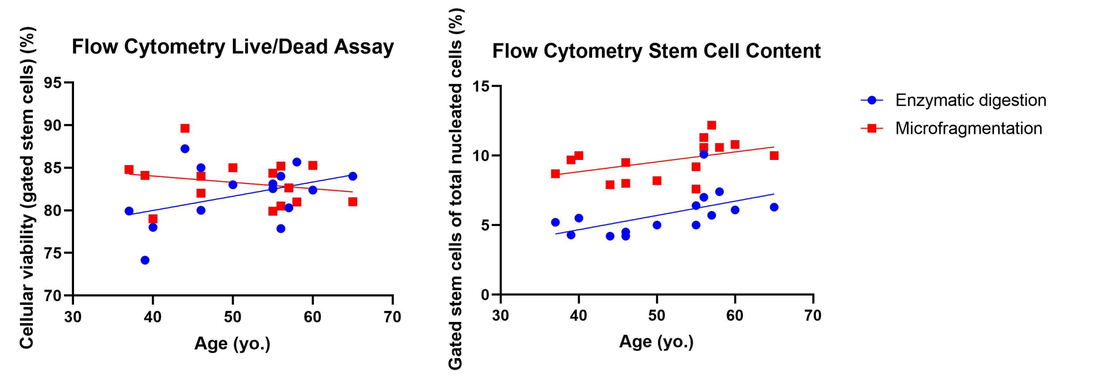


Figure 4: *Flow cytometry results on viability (%) and yield of stem cells out of total nucleated cell count (%) from abdominal adipose tissue.*

CONCLUSION

Microfragmentation is a promissing method to process clinically relevant stem cells from abdominal adipose tissue for the treatment of osteoarthritis.

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The Danish Rheumatism Association

[A] Kohli N, Al-Delfi IRT, Snow M, *et al.* CD271-selected mesenchymal stem cells from adipose tissue enhance cartilage repair and are less angiogenic than plastic adherenct mesenchymal stem cells. Sci Rep.; 2019;9:1-12.
[B] Barfod KW, Blønd L. Treatment of osteoarthritis with autologous and microfragmented adipose tissue. Dan Med J. 2019;66:1-5.
[C] Bianchi F, Maioli M, Leonardi E, *et al.* A new nonenzymatic method and device to obtain a fat tissue derivative highly enriched in pericyte-like elements by mild mechanical forces from human lipoaspirates. Cell Transplant; 2013;22:2063-77.