Fishing nucleus pulposus progenitor cells from bovine intervertebral discs using three different sorting methods

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INTRODUCTION

- Nucleus pulposus progenitor cells (NPPC) were recently described as Tie2+ cells (angiopoietin receptor) in human, mouse and bovine tissue and to possess multi lineage differential potential.[1,2]
- NPPC might theoretically represent an outstanding cell source for regeneration of the intervertebral disc (IVD).[3]
- However, isolation of these cells is still cumbersome and suitable culture conditions for maintenance of NPPC are yet unknown.

Study Aim

Investigate and comparison of three isolation methods for NPPC isolation from bovine NP cells.

MATERIALS and METHODS

NPPC Cell Sorting

- Bovine NP cells were isolated using a two step digestion protocol from one-year old animals.[4]
- NP cells were incubated with primary antibody (AB) against Tie2 (Bioss Antibodies).
- Sorting of NPPC using FACS (Fluorescence-activated cell sorting), pluriSelect (non-magnetic selection by size) or MACS (Magnetic-activated cell sorting).

Output Parameter

- NPPC yield as percentage of total cell number.
- Colony count after 10 days in MethoCult™ cellulose medium.

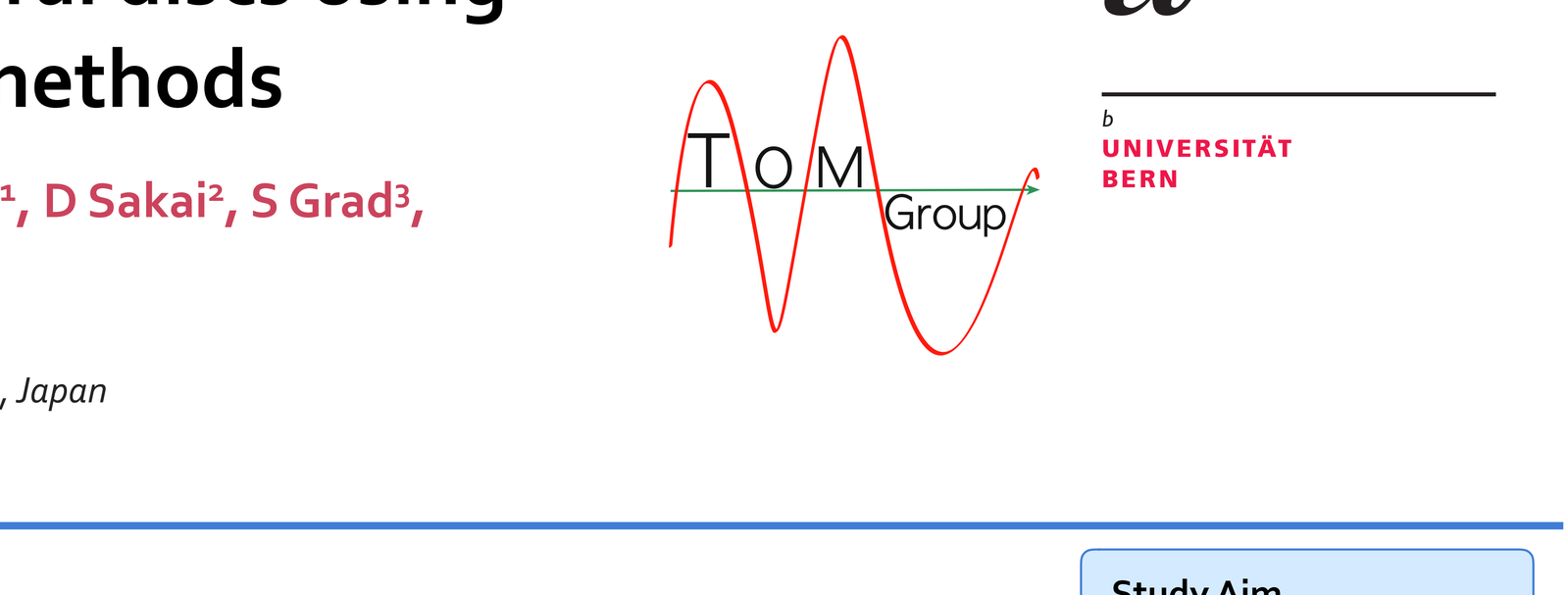

Figure 1. Isolation of Tie2+ cells using FACS, pluriSelect and MACS.

RESULTS

- FACS yielded the highest percentage of Tie2+ cells.
- MACS and pluriSelect are faster methods but less efficient.
- pluriSelect is the least invasive method and results in cells without any bead or fluorescent AB attached.

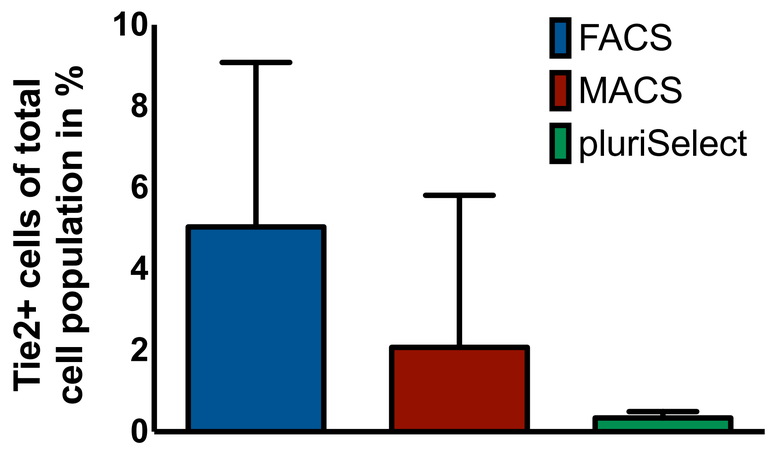

Figure 2. Percentage of Tie2+ cells relative to total NP cell population using FACS (n=9), MACS (n=4) and pluriSelect (n=2) (mean ± SD).

CFU Assay

- FACS Tie2 sorted cells produced a higher number of colonies for both the positive and negative cells.
- For both sorting methods a non-significant difference between Tie2 positive and negative cells could be observed.

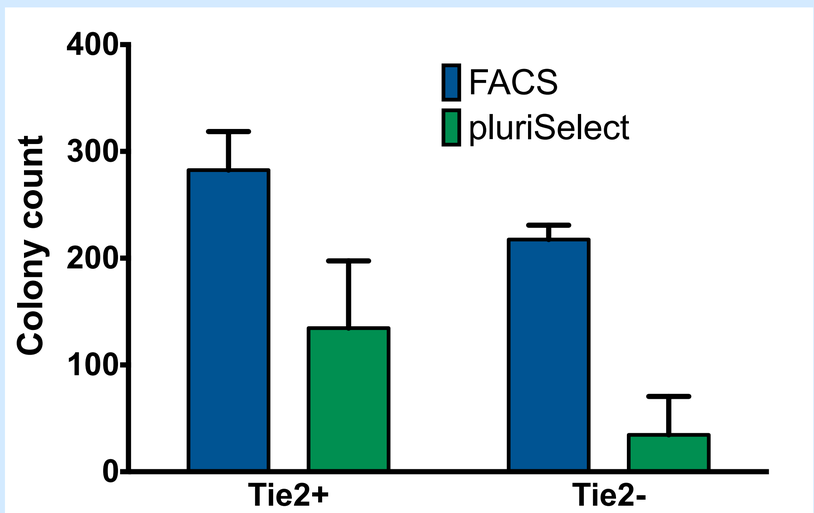

Figure 3. Numbers of colonies for Tie2 positive and negative cells sorted either by FACS (n= 6) or pluriSelect system (n=2) (mean ± SD).

CONCLUSION

- NPPC can be successfully isolated from bovine NP tissue with all three methods.
- Cell yields differ among sorting methods: FACS > MACS > pluriSelect.
- The difference in cell yield of FACS to the other methods might be explained by too generous gating.
- Both, MACS and pluriSelect, offer fast and selective NPPC sorting alternatively to FACS.

QR Code

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