Comparison of methods for human adipose-derived stem cells (hADSCs) differentiation into neural crest cells (NCCs)

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Introduction

The aim of this study was to compare the effectiveness differentiation of human adipose-derived stem cells (hADSCs) into neural crest cells (NCCs) of two independent methods for their subsequent use in ophthalmological transplants.
hADSCs were cultured in basal medium (Cadenas-Martin et al., PMID: 34769411)[1] and then, specially selected culture media (Ali et al., PMID: 29847650)[2] and (Wagoner et al., PMID: 29685994)[3] were introduced to boost their differentiation into NCCs. Furthermore, the culture plates were coated with laminin or vitronectin, to further enhance efficiency of differentiation. Cells were cultured in Ali medium for 11 days or 18 days in the Wagoner medium, following preliminary results[1]. Specific NCC gene expression analysis was performed by qRT-PCR (quantitative reverse transcription polymerase chain reaction) for genes SOX10, CXCR4, Noggin, PAX3, NGFR and Nestin, and immunofluorescence experiments were performed using antibodies characteristic for NCCs: CXCR4 and Nestin and imaged with confocal microscopy. The experiments were repeated three times and analyzed using one-way ANOVA with the Bonferroni's post hoc test.

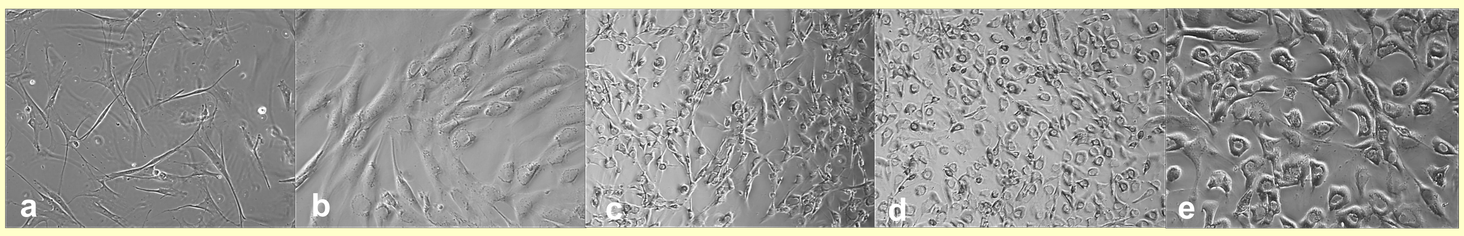


Figure 1. hADSCs cell differentiation into NCSCs on plate coated with laminin a) 24 h in basal medium, b) 4 days in Ali medium, c) 7 days in Ali medium, d) 9 days in Ali medium, e) 11 days in Ali medium.

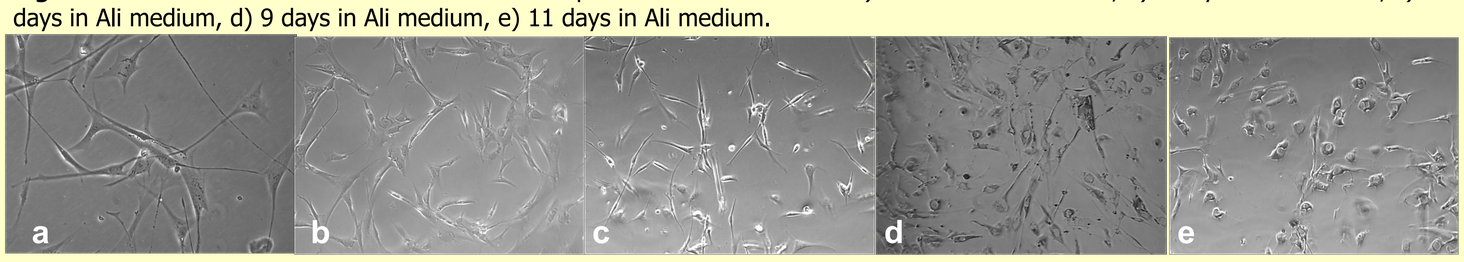


Figure 2. hADSCs cell differentiation differentiation into NCSCs on plate coated with vitronectin a) 24 h in basal medium, b) 4 days in Ali medium, c) 7 days in Ali medium, d) 9 days in Ali medium, e) 11 days in Ali medium.

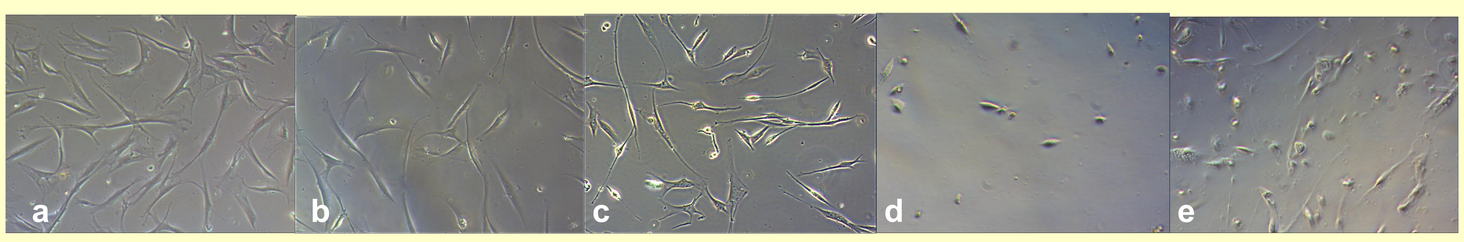


Figure 3. hADSCs cell differentiation into NCSCs on plate covered with laminin a) 24 h in basal medium b) 4 days in Wagoner medium, c) 7 days in Wagoner medium, d) 14 days in Wagoner medium, e) 16 days in Wagoner medium.

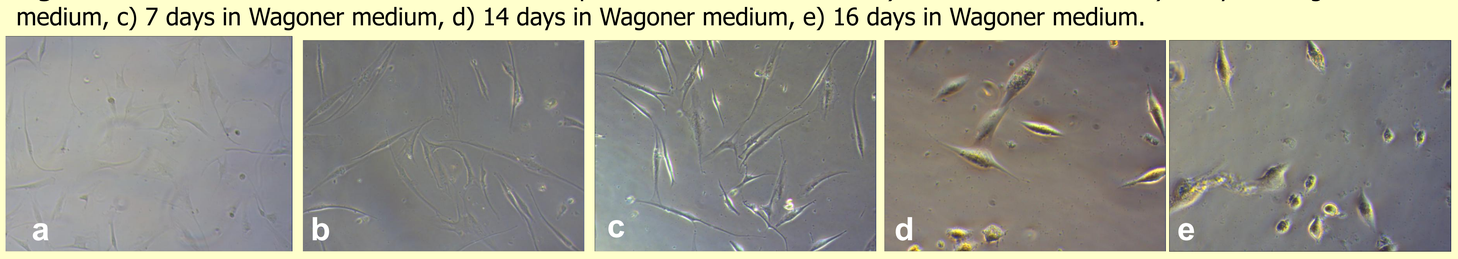


Figure 4. hADSCs cell differentiation into NCSCs on plate coated with vitronectin a) 24 h in basal medium, b) 4 days in Wagoner medium, c) 7 days in Wagoner medium, d) 14 days in Wagoner medium, g) 16 days in Wagoner medium.

Inverted Light microscopy

The pictures on Figures 1-4 show the time-dependent course of cell differentiation (phase contrast light microscopy) starting from hADSCs and ending with NCCs using 2 different plate coatings and Ali and Wagoner media.

Immunofluorescence and confocal microscopy

After cell fixation, immunofluorescence experiments were performed using antibodies characteristic for NCCs: CXCR4 and Nestin, and imaged with confocal microscopy. The overall results of immunofluorescent experiments are presented on Figure 5.

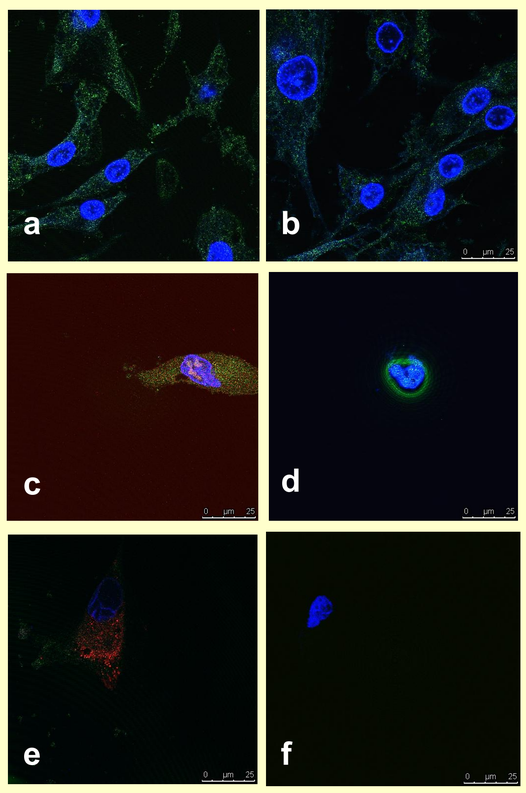


Figure 5. Immunofluorescence experiments: hADSCs cell differentiation into NCCs (day 11, plate with Ali medium and laminin) and a) CXCR4 antibody b) nestin; Wagoner medium + laminin and c) CXCR4, d) nestin, Wagoner medium + vitronectin and e) CXCR4, f) nestin

qRT-PCR gene expression analysis (quantitative reverse transcription polymerase chain reaction)

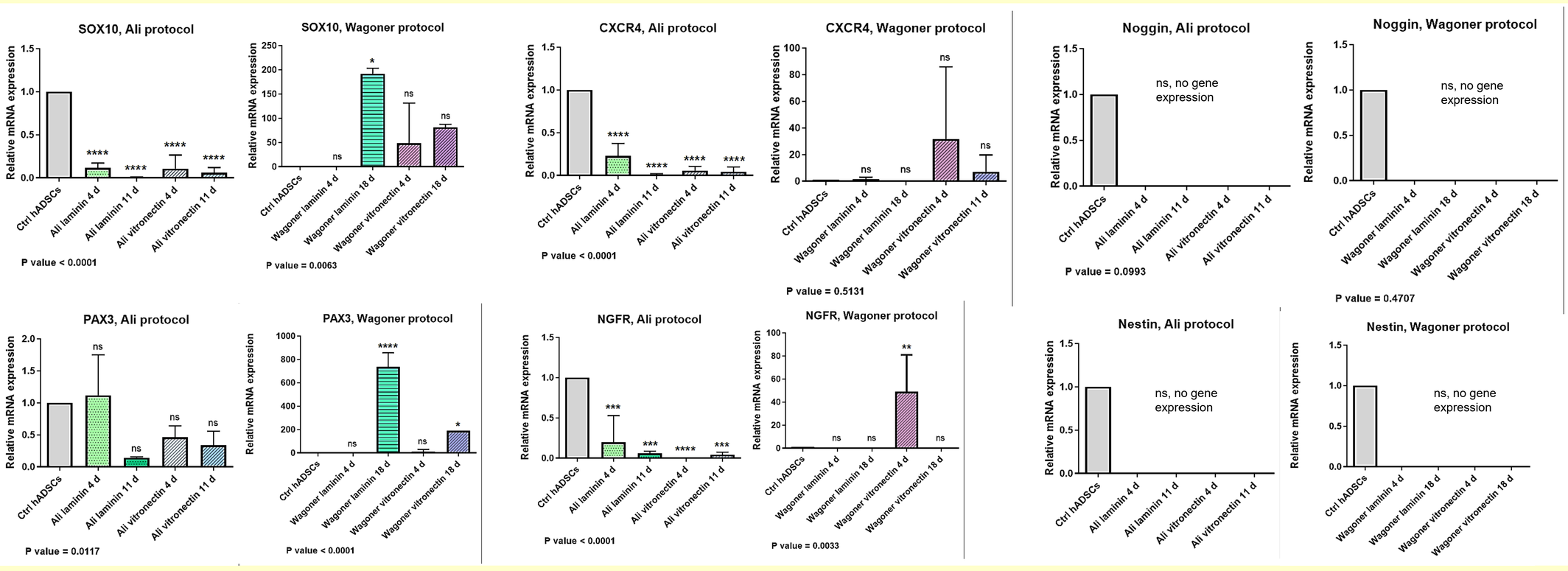


Figure 6. Expression of SOX10 CXCR4, Noggin, PAX3, NGFR and Nestin mRNA, in hADSCs differentiaed to NCSCs at day 4 and 11/18, regarding Ali/Wagoner laminin/vitronectin protocol, respectively, measured by qRT-PCR. β-actin was used as a housekeeping control gene. Data are presented as the means ± SD. Values were normalised to β-actin (ns, not significant;*, $p \leq 0.05$; **, $p \leq 0.01$; ***, $p \leq 0.001$; ****, $p \leq 0.0001$; one-way ANOVA with Bonferroni's post hoc test in reference to control probe with hADSCs (donor EU16, passage 3).

Conclusions

The obtained results allowed us to select an appropriate protocol for future research. Both protocols yielded NCCs, but with different levels of NCC gene expression. Experiments conducted in Ali medium produced faster results and better cell growth rate. However, the level of gene expression characteristic for NCCs was significantly higher in the Wagoner medium, as confirmed by immunofluorescence experiments.

Bibliography

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2. Ali M, Khan SY, Vasanth S, et al., *Invest Ophthalmol Vis Sci.*, **2018**, 59(6):2437-2444.
3. Wagoner MD, Bohrer LR, Aldrich BT, et al., *Biol Open.*, **2018**, 7(5):bio032102.

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