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Differentiation of co-cultured human adipose-derived stem cells

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Purpose: To evaluate differentiation of co-cultured human adipose-derived stem cells (hASCs) with keratocytes *in vitro*

Material and Methods: We developed a co-culture system using Costar transwell inserts to grow hASCs on bottom and keratocytes on top in keratocyte differentiating medium (KDM). hASCs that were cultured in complete culture medium (CCM) and KDM were used as control. After 16 days, hASCs were examined for morphology and proliferation by cell count. qRT-PCR and flow cytometry were used to detect the expression of aldehyde dehydrogenase 3 family, member A1 (ALDH3A1) and keratocan.

Results: hASCs became more dendritic and elongated in co-culture system relative to CCM and KDM. They started to grow slower as differentiation progressed. qRT-PCR showed a definite trend towards increased expression of both ALDH3A1 and keratocan in co-culture system despite statistically non-significant p-values. However, flow cytometry showed significantly increased protein level of ALDH3A1 and keratocan in co-culture system relative to CCM group ($p<0.0001$) and even relative to KDM group ($p<0.0001$ for ALDH3A1 and $p<0.01$ for keratocan)

Conclusion: Co-culture system is a promising method of stem cell culture to induce differentiation before in vivo applications. Our study reveals an important potential for bioengineering of corneal tissue using autologous multi-potential stem cells

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