

## Isolation and Partial Characterization of Human Amniotic Epithelial Cells: The Effect of Trypsin

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### Abstract

**Background:** Despite the extensive information available in the literature, cell surface marker signature of human Amniotic Epithelial Cells (hAECs) remains controversial. The aim of the present study was to characterize immunophenotypic features, proliferative capacity and immunogenicity of hAECs. We also tested whether expression of some cell surface markers is influenced by the type of trypsin used for tissue digestion.

**Methods:** Single cell suspensions of amniotic membranes from four human placentas were isolated by enzymatic digestion and expression of CD9, CD10, CD29, CD34, CD38, CD44, CD45, CD73, CD105, CD133, HLA-I, HLA-DR, HLA-G, SSEA-4, STRO-1 and OCT-4 was then evaluated by flow cytometry. The differential impact of four trypsin types on the yield and expression of CD105 and HLA-I was also determined. The proliferative capacity of cultured hAECs was assessed and compared in the presence and absence of Epidermal Growth Factor (EGF). To test their immunogenicity, hAECs were injected into Balb/c mice and the reactivity of hyperimmunized sera was examined by immunofluorescence staining.

**Results:** Nearly all purified cells expressed mesenchymal markers, CD9, CD10, CD29, and CD73 and the embryonic marker, SSEA-4. A large proportion of the cells also expressed STRO-1 and OCT-4. The purified cells also expressed HLA-G and HLA-I. A very small proportion of hAECs expressed CD34, CD38, CD44, CD133 and HLA-DR. The type of trypsin used for enzymatic digestion affected both the percentage and expression of HLA-I and CD105. hAECs revealed substantial proliferative capacity only when cultured in the medium supplemented with EGF. These cells were shown to be capable of inducing high amounts of anti-donor antibodies.

**Conclusion:** Here we provided evidence that hAECs are immunogenic cells with high level of HLA-I expression. Furthermore, this work highlighted the impact of isolation procedure on the immunophenotype of hAEC.

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**Keywords:** Cell proliferation, Epithelial cells, Immunophenotyping, Placenta, Stem cell, Trypsin

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### Introduction

Human placenta is composed of three layers: amnion, chorion, and decidua. Amnion

is derived from the embryo and suggested to