Human Amniotic Epithelial cells as an alternative source of Human Embryonic Stem cells

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Introduction:

Human Amniotic Epithelial Cells isolated from Human Amniotic membrane and Human Amniotic Fluid from term delivery express surface markers normally present on embryonic stem cells and germ cells. The Human Amniotic Stem Cells(HAMSC)express pluripotent stem cell-specific transcription factors Octamer-binding protein 4(Oct-4) and nanog. The HAMSCs retained stem cell characteristics forming spheroid bodies when maintained in Celprogen's human amniotic stem cell undifferentiating complete growth medium and extracellular matrix which does not require mouse feeder layers. The HAMSCs have the potential to differentiate to three germ layers: endoderm (pancreas), mesoderm (cardiomyocyte) and ectoderm (neural cells) as confirmed by immunocytochemistry and genetical markers specific for each cell type. The HAMSCs obtained after birth from amniotic membrane and amniotic fluid may provide as an alternative source for human stem cells for regenerative and transplatation medicine. The HAMSCs transdifferentiation into islet of Langerhan insulin producing Beta cells, neuronal cells and functional cardiomyocytes may provide alternative sources of stem cell therapies for patients that may seek transplation of these specific stem cells. The HAMSC are able to undergo transdifferetiation into the three germ layers invitro utilizing Celprogen's neuronal stem cell, pancreatic and cardiomyocyte differentiation Kits. The HASCs were examined for tumorigenicity in SCID mice and no observation of tumor formation up to 6 months of observations in SCID mice.

Method:

Human Amniotic fluid and membrane were obtained from five consented individuals after term delivery. The amniotic fluid and membrane were processed utilizing Celprogen method to obtain human embryonic stem cells that were pre-selected as cells that expressed embryonic stem cell markers Oct-4, nanog, stage specific embryonic antigen 4 /3 (SSEA-4/3), Antibody to a specific extracellular matrix molecule is synthesized by undifferentiated stem cells(TRA-1-60), Antibody to a specific extracellular matrix molecule is synthesized by undifferentiated stem cells(TRA-1-81) & Alkaline Phosphatase (AP) in culture. Two groups of five cultures were prepared in 6 well pre-coated tissue culture flasks with Celprogen’s human embryonic stem cell extracellular matrix one set of five cultures were from human Amniotic fluid and membrane and were cultured in

5 of the 6 well tissue culture flasks (experimental set Group A). The other set were from human Embryonic stem cells (HES)that were cultured in 5 of the 6 well tissue culture flasks (control set Group B). The stem cells in both groups were maintained in Celprogen Human Embryonic Stem Cell Complete Growth and Differentiation medium and Matrix. The experiment design for differentiation was as follows:

Cultured flask # (6 well flask)	Group A (Human Amniotic fluid / membrane stem cells)	Group B (Human Embryonic Stem Cells)
1	Characterized for stem cell markers	Characterized for stem cell markers
2	Genetic profile for stem cells marker	Genetic profile for stem cells marker
3	Differentiated into neuronal cells with Celprogen media and matrix kit for neuronal differentiation and expansion	Differentiated into neuronal cells with Celprogen media and matrix kit for neuronal differentiation and expansion
4	Differentiated into Pancreas with Celprogen media and matrix kit for Pancreas differentiation and expansion	Differentiated into Pancreas with Celprogen media and matrix kit for Pancreas differentiation and expansion
5	Differentiated into cardiomyocyte with Celprogen media and matrix kit for cardiomyocyte differentiation and expansion	Differentiated into cardiomyocyte with Celprogen media and matrix kit for cardiomyocyte differentiation and expansion

Results:

The results for the immunological profile with the stem cell markers are tabulated in table 1 below.

Table 1: Stem marker profile for the HAMSCs and HES

Stem Cell Markers	Group A	Group B
AP	v	v
SSEA 3/4	v	v
TRA-1-60	v	v
TRA-1-81	v	v
Oct-4	v	v

The Genetic profile data for Differentiation into following germ layers is presented in the following figures 1-6 below:

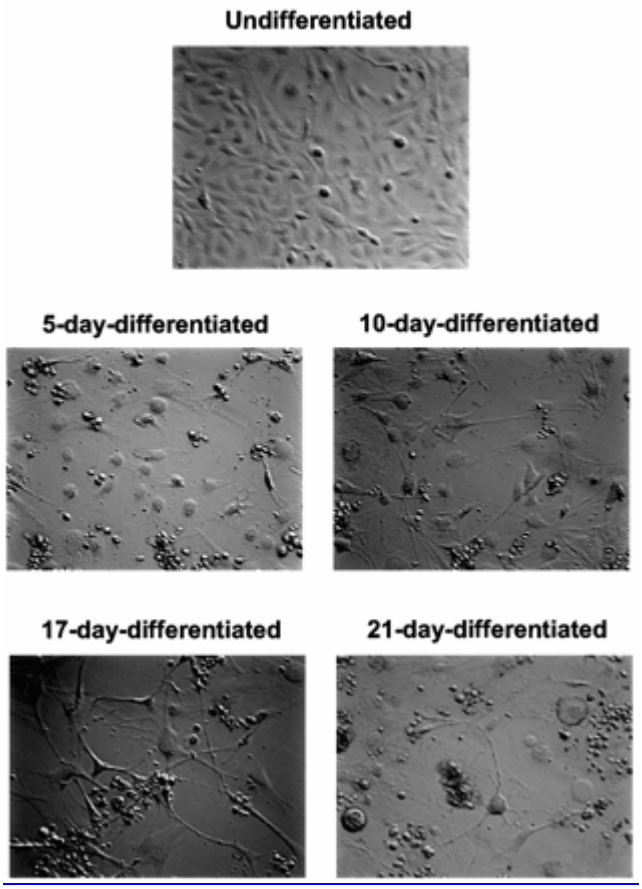

Figure 1: neuronal differentiation of HASC with Celprogen Neuronal Differentiation medium (serum free) and matrix.

Figure 3: HASC in Celprogen's Un-differentiated medium and matrix (light microscopy)

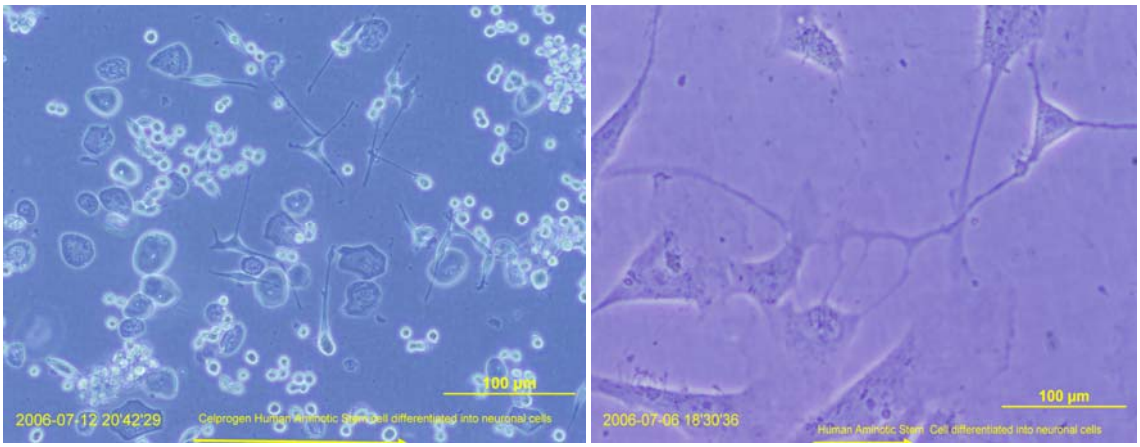

Figure 4: HASC in Celprogen's neuronal differentiation medium and matrix (light microscopy).

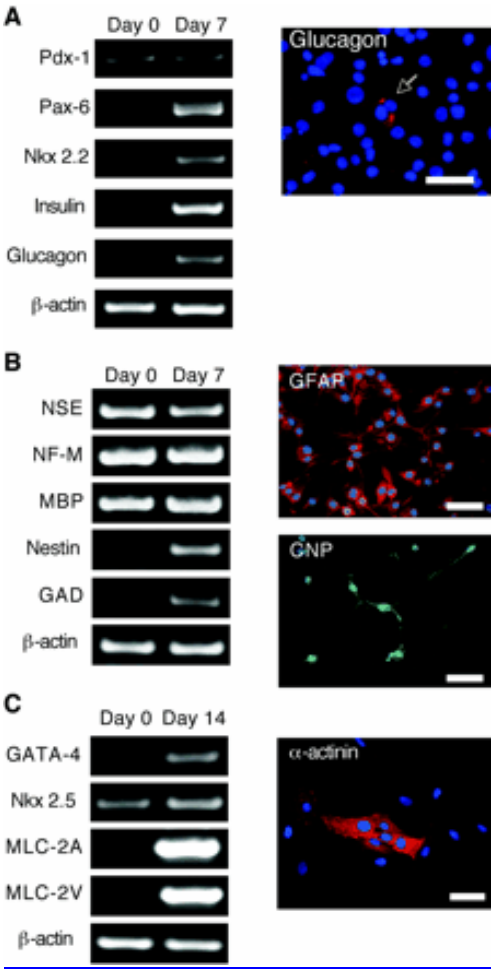

Figure2: A. Differentiation of HASC into pancreatic cells.
B. Differentiation of HASC into neuronal cells.
C. Differentiation of HASC into Cardiomyocyte cells.
The differentiation was performed with Celprogen's Differentiation kits for each specific cell type and matrix combination in serum free medium.

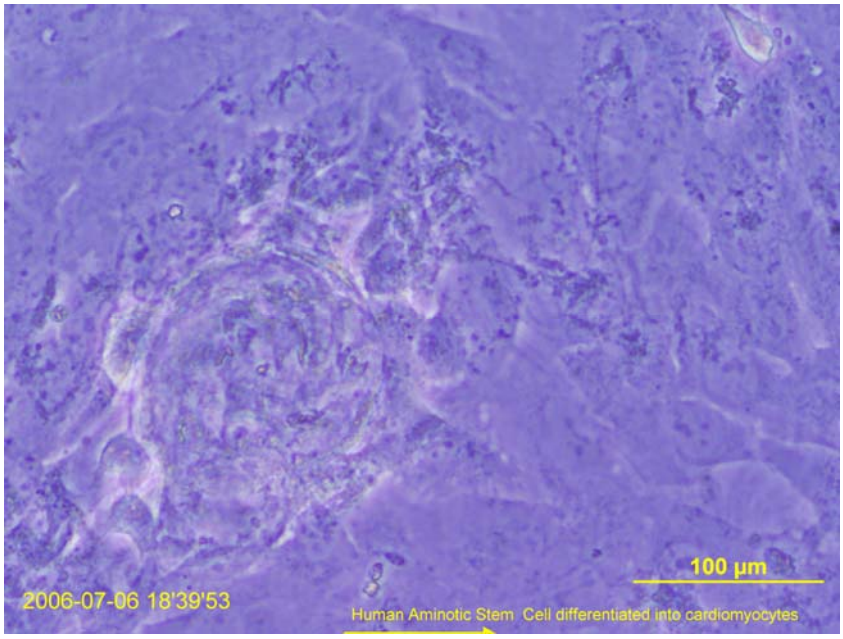

Figure 5: HASC in Celprogen's cardiomyocyte differentiation medium and matrix.

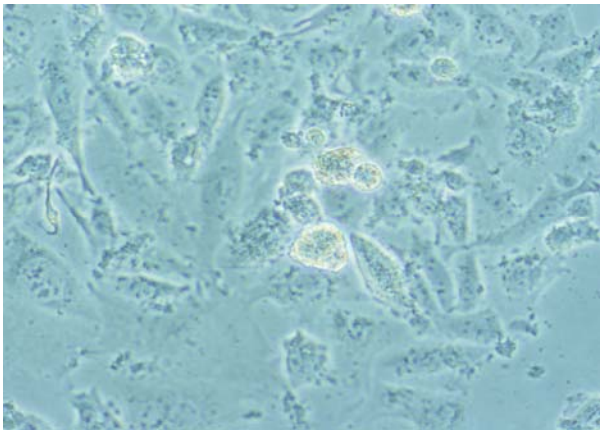
Figure 6: HASC in Celprogen's Pancreatic differentiation medium and matrix.

Conclusion:

From this study, we have demonstrated the possibility of pre-selecting and differentiating HAMSCs in vitro into pancreatic cells, cardiomyocytes, and neuronal cells. This provides the ex-vivo differentiation and expansion of HAMSCs to functional, neuronal tissue, cardiomyocyte and islets of Langerhan Beta cells into patients with physiological impaired, cardiomyocytes, neuronal disorders and islets of langerhan cells. Further pre-clinical studies need to be performed prior to introducing a successful stem cell based therapy for the clinic.

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