Continuous Collection of Stem Cells from a Human Placenta Perfusion Co-Culture

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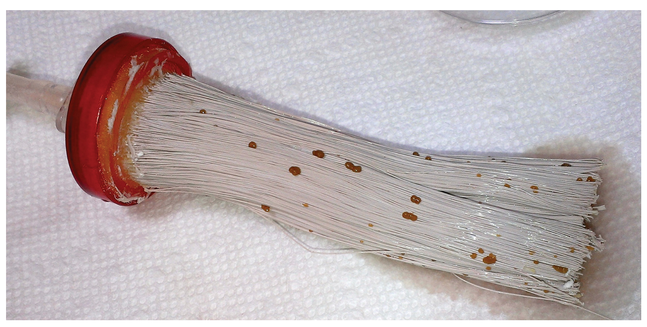
INTRODUCTION

The human placenta represents an attractive source of stem cells as it is readily available, provides large numbers of cells and presents low ethical constraints. The potential for human placenta-derived cells to produce stem cells in a hollow fiber bioreactor co-culture system was investigated.



METHODS

Full term human placentas were sourced from the National Disease Research Interchange with approved protocols and consent. The intubated and PBS perfused placenta was collagenase treated (Worthington Biochemicals, Lakewood, N.J., U.S.A.) for one hour followed by harvest using a FiberCell Systems Duet® pump (perfusate). Perfusate cell mass was cleared of red cells, washed once in fresh cell culture medium and inoculated into a 5kd MWCO polysulfone hollow fiber bioreactor cartridge for culture (FiberCell Systems Cat# C2008). 125 mL of medium was replaced every 4 days. The extra-capillary space (ECS0 was flushed on day 2 and day 4 to remove non-adherent cells. Cells were harvested from the ECS on day 7 and placed in T25 flasks for subculture. Samples were taken from the ECS for flow cytometry and scored. By day 10 red masses of cells were observed on the surface of the fibers. On day 28 the cartridge was cut open and the cell masses harvested for microscopy

Frozen and OCT embedded samples of the red masses were prepared to slides at Alizée Pathology, LLC, Thurmont, Md. The specimens were cut at 5 microns and stained with H&E and Masson's Trichrome and immunostained for c-Kit, CD31, Cytokeratin, and vimentin.

RESULTS

Cells harvested from the ECS after 7 days of culture showed a mixed phenotype by flow analysis but demonstrated 18% OCT3/4 expression, an indication of pluripotency. When these harvested cells were placed in T25 flasks, after 5 days showed 99% mesenchymal stem cell markers along with the presence of embryoid bodies in the flasks.

	INITIAL BLOOD FRACTION	PERFUSATE	PLACENTAL DIGEST	ECS HARVEST
VIABLE CELLS	2 X 10^8	2 X 10^9	1.5 X 10^9	2-3 X 10^7*
CD45	90	77	17	4
CD34	16	27	16	0
CD133/2	9	10	3	2
CD31	63	71	29	3
CD13	71	80	82	6
CD105	22	34	39	43
CD73	26	42	53	18
CD90	6	6	47	5
CD14	14	25	70	23
NANOG	-	-	-	0
OCT3/4	-	-	-	18

*On average, mean viability 88%, mean diameter 8.2 microns.

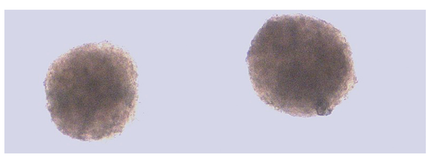
TABLE 1. Enumeration of cell number and phenotype prior to loading cartridges and of cells collected from ECS every 3-7 days.

PHENOTYPE	ECS HARVESTED	FLASK CULTURED
CD45	4%	1%
CD34	0%	0%
CD133/2	2%	0%
CD31	3%	48%
CD13	6%	83%
CD105	43%	99%
CD73	18%	99%
CD90	5%	96%
CD14	23%	4%
NANOG	0%	0%
OCT3/4	18%	13%

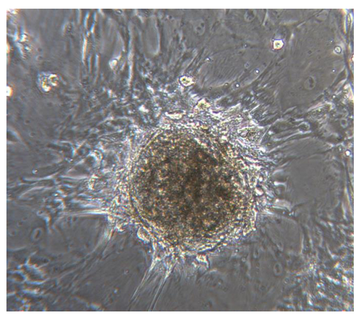
TABLE 2. Direct cartridge ECS-harvested cells vs. those post 3-5 day flask culture

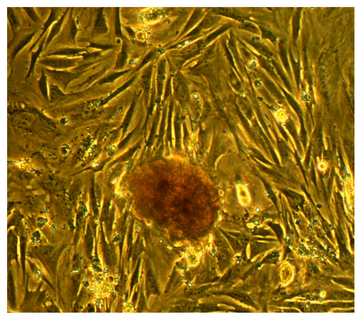
DISCUSSION

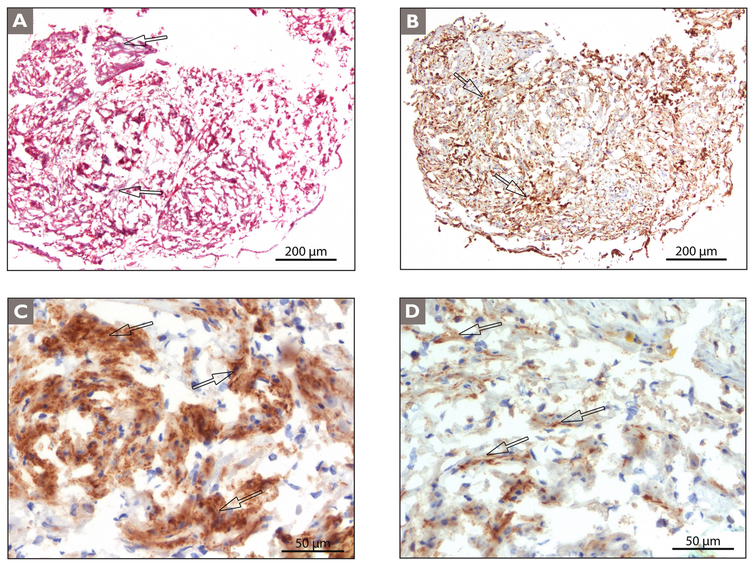
The presence of smaller cells in the ECS harvests, OCT3/4 phenotype and the highly proliferative nature of this harvest when placed into flasks suggests the presence of very small embryonic-like stem cells. The hollow fiber cell culture environment is clearly different than flask culture. Three dimensional structures were formed on the surface of the fibers. Cytokine concentration and cell to cell interactions may play a role in their formation.



When harvested cells were placed into flasks, the different cell culture environment induced these cells to differentiate into mesenchymal stem cells and generate spheroids. The recapitulation of the placental construct may represent a unique source for continuous culture and collection of stem cells. Hollow fiber bioreactors represent a different cell culture environment with resultant differences in cell phenotype from flask culture. These experiments demonstrate the capability of hollow fiber bioreactors to support continuous production of certain stem cell types in a single use system. Larger scale systems have the potential to produce stem cells on a continuous basis and meet the criteria for their bio-manufacturing in a single-use system on a clinical scale.







A: There is a complex tissue architecture of the cellular cluster characterized by a mixture of epithelioid and mesenchymal cells supported by a tenuous extracellular matrix (arrow). Fine cellular structure is blurred by ice crystal artifact.
B: Relatively widespread staining of the mesenchymal cellular population for intermediate filaments (Vimentin) with more densely cellular clusters showing high intensity signal (arrow)

C: Intense cytoplasmic c-kit (CD117) staining for stem cell factor receptor. **D:** CD31 positive cells appear to be streaming along the extracellular matrix (arrows) suggesting possible angiogenic differentiation. The CD31 signal appears membrane-bound although freezing artifacts preclude definitive confirmation.