

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

John J.S. Cadwell, FiberCell Systems Inc., Frederick, Md. 21701 U.S.A., and James C. Hardy, Hemacell Perfusion, Inc. Frederick, Md. 21701 U.S.A.

## 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 placental derived cells to produce stem cells in a hollow fiber bioreactor co-culture stem cell production was investigated.



## METHODS

Full term human placentas were sourced from National Disease Research Interchange

with approved protocols and consent. The intubated and PBS perfused placenta was collagenase treated 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 (ECS) was flushed on days 2 and day 4 to remove non-adherent cells. Cells from the day 7 flush were placed into T25 flasks for sub-culture. Samples were taken 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



## RESULTS

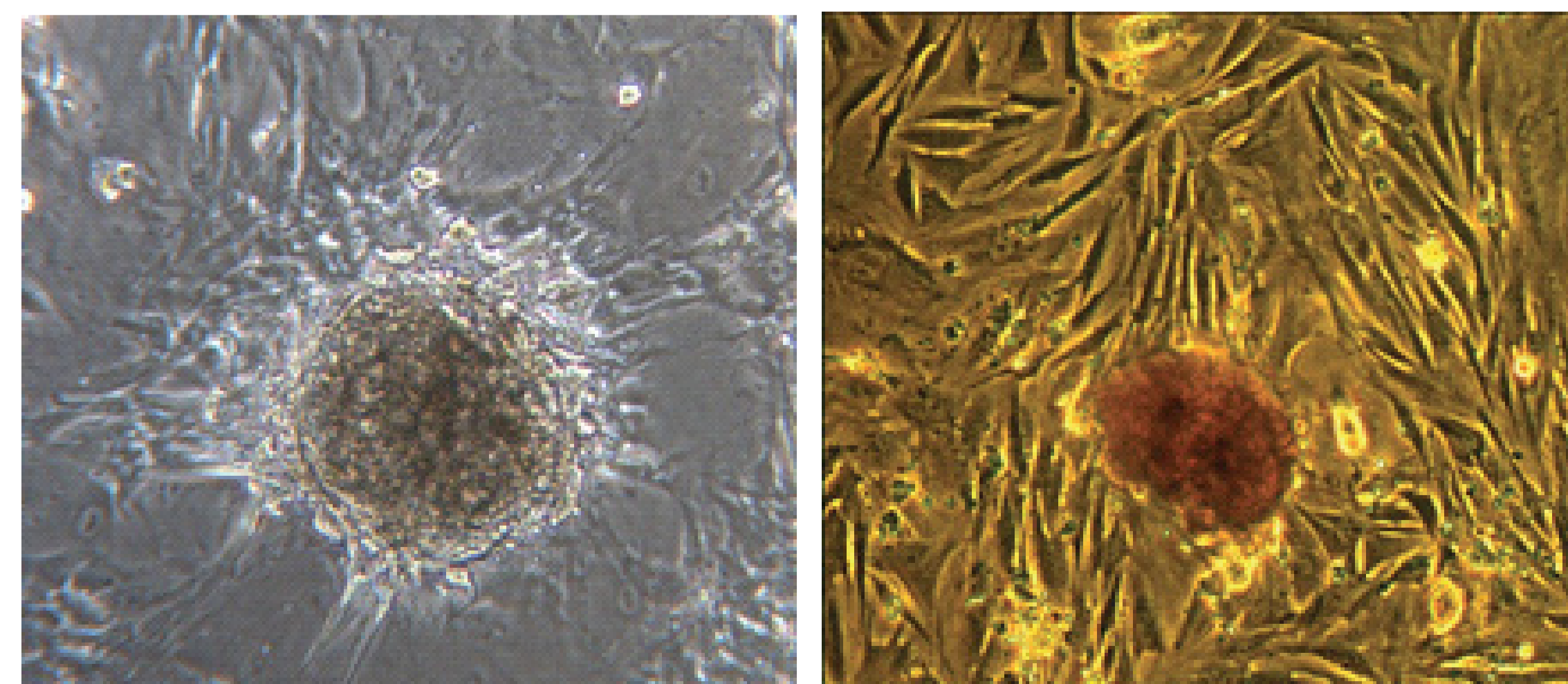
Cells harvested from the ECS after 7 days of culture showed a mixed phenotype by flow analysis but demonstrated 18% OCT3/4 expression, an indication of pluripotency. When these harvested cells were placed in T25 flasks, after 5 days showed 99% mesenchymal stem cell markers along with the presence of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

	INITIAL BLOOD FRACTION	PERFUSATE	PLACENTAL DIGEST	ECS HARVEST
VIABLE CELLS	2 X 10 <sup>8</sup>	2 X 10 <sup>9</sup>	1.5 X 10 <sup>9</sup>	2-3 X 10 <sup>7</sup>
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

PHENOTYPE	ECS HARVESTED	FLASK CULTURED
CD45	4%	1%
CD 34	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 1.** Enumeration of cell number and phenotype prior to loading cartridges and of cells collected from ECS every 3-7 days. The last two rows represent the "percent positive" for the indicated phenotype.

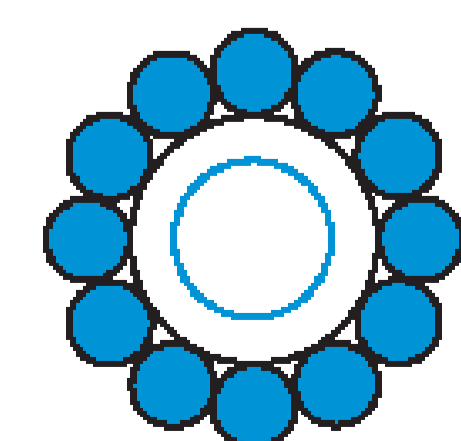
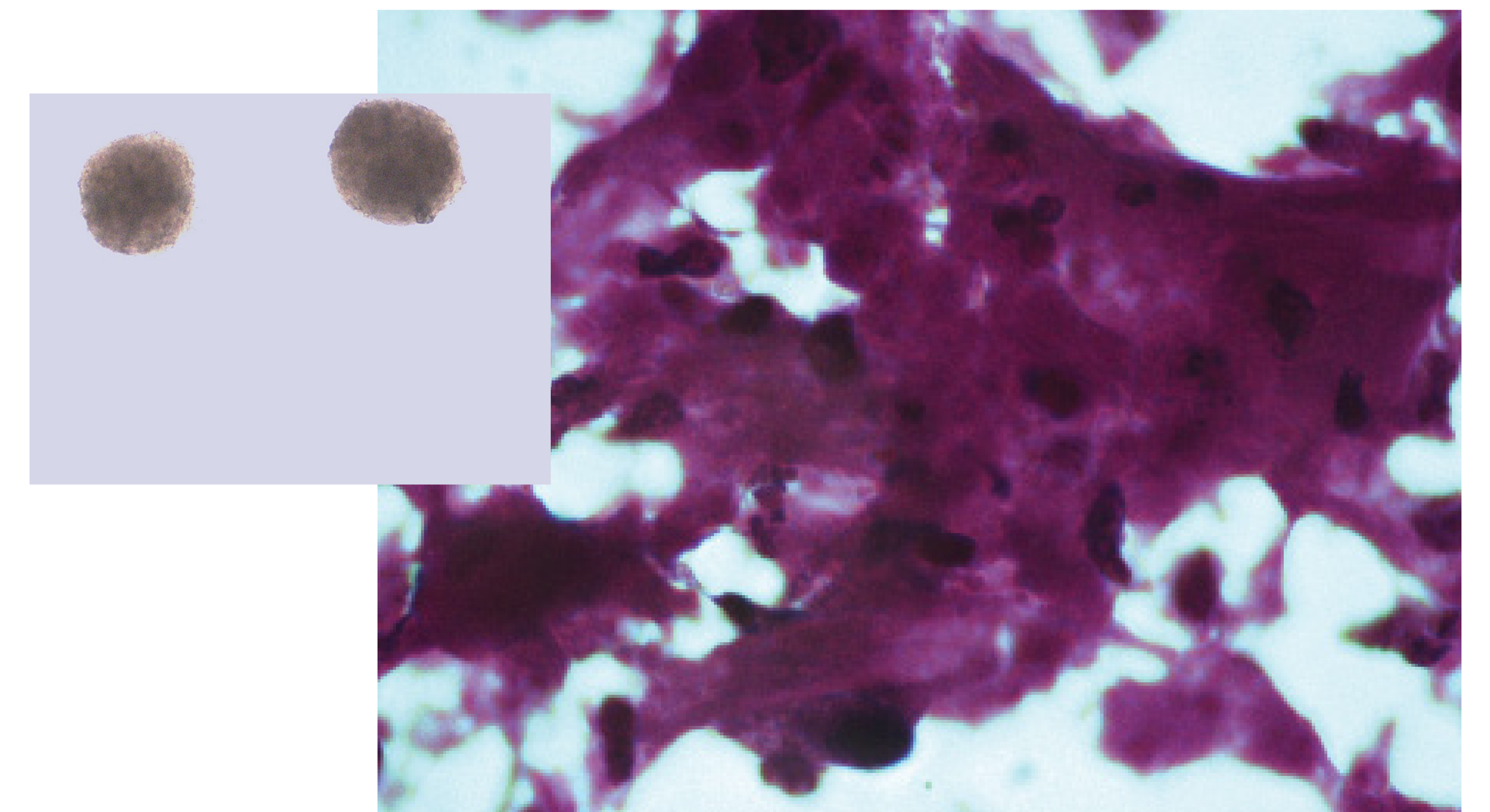
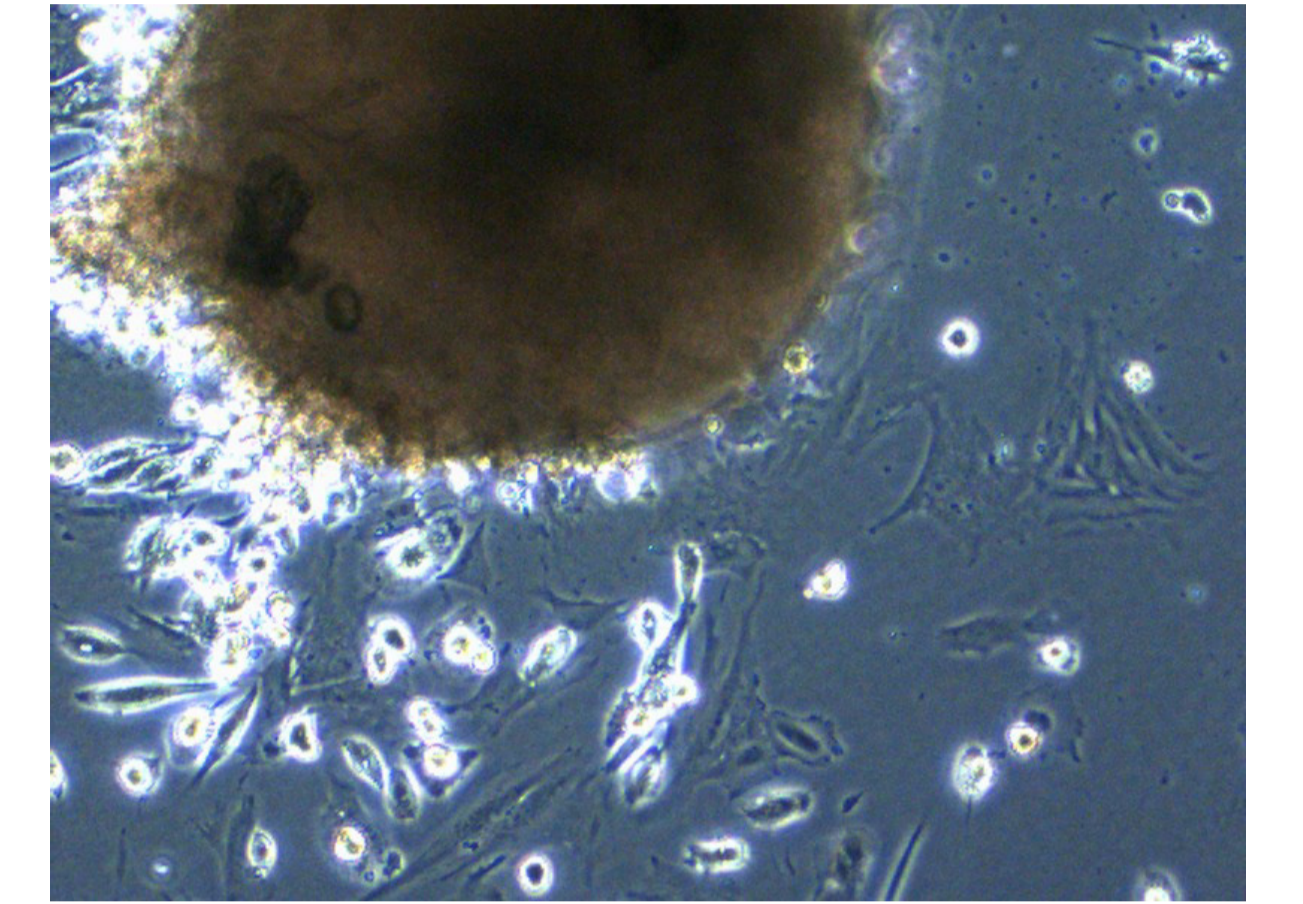
**TABLE 2.** Direct cartridge ECS-harvested cells vs those post 3-5 day flask culture



## 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 a type 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.



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