



Culture of Placental Derived Cells in a Hollow Fiber Bioreactor Cartridge

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Interest in the area of stem cell research and culture methods has grown significantly in the past 5 years. There are many different cell types and sources of stem cells available. The desired endpoints are as varied as the number of sources and types of cells. One source of stem cells gaining interest is the human placenta. Although the cell population of this source is somewhat undefined, the human placenta is not restricted by the ethical considerations that surround the use of embryonic stem cells and have been shown potential to generate blood forming cells as well as hepatocytes, neurons, and chondrocytes.

Current protocols for the collection of mesenchymal stem cells from placenta entail a perfusion step to harvest hematopoietic stem cells in the cord/placental circulation loop and to remove red cells followed by a digestion with collagenase or other enzyme. Typically these cells are then placed into T flasks for culture over a period of several weeks. During this time the medium is changed on a regular basis. The medium changes aid in flushing out the non-adherent cells and debris and removes them from the plastic adherent cells. The adherent cells are mesenchymal stem cells derived from the placenta.

This focus of this application is to replace the flask culture step with a culture of the mixed population of cells in the hollow fiber bioreactor cartridge.

Preliminary data had shown a definite increase in glucose uptake rate when the 5kd MWCO cartridge was used instead of the 20kd MWCO cartridge. It was thought that perhaps the smaller pore size of the 5kd cartridge retained important cytokines that facilitated cell-to-cell interactions.

Protocol

A normal intact human placenta was sourced from a local hospital under conditions approved by the Institutional Review Board (IRB). The umbilical vein was cannulated and a solution of PBS was perfused for a time of one hour. Perfusion was performed utilizing the Machine Pulsatile Perfusion method described elsewhere. Blood forming hematopoietic stem cells and other components were collected from the perfusate. This also reduced the red cell burden. After one hour at

room temperature the solution was changed to PBS containing one gram per liter of collagenase (Worthington Biochemicals, Lakewood, New Jersey). This was perfused for 2 hours at room temperature. The placenta was digitally disaggregated and the cells were collected, washed once in cell culture medium and then the red cells removed using ACK lysing buffer (Quality Biologicals catalog number 118-156-101) per manufacturers instructions.

Cells were washed once more in cell culture medium. Total number of viable cells was difficult to establish due to the significant amount of cell debris and red cells still present. 20 ml of the cell isolate (out of a total of 40 ml) was seeded into a FiberCell® Systems C2008 5kd MWCO polysulfone cartridge and the culture initiated with 100 ml of DMEM/F12 + 10% FBS and 2% pen/strep at a flow rate of 60 ml/minute.



Figure 1 Machine Pulsatile Perfusion of the placenta.



Figure 2 the placenta is supported by a stainless steel screen during digestion.

On day 2, day 3 and day 5 the ECS (extracapillary space) was washed with cell culture medium to remove any non-adherent cells. Starting with day 10 the ECS was harvested, photographed under a microscope and plated into 6 well dishes twice a week. The cartridge remained viable for over three months of continuous culture. The culture was terminated at that time.

Results

The initial glucose uptake rate was around 100 mg/day. By day 10 this had increased to a level of 250-350 mg/day where it remained in a fairly steady state throughout the culture period. As the harvests progressed the amount of cell debris was reduced. Significant cell numbers were extracted from the cartridge and most interestingly clusters of cells that resembled embryoid bodies, in good numbers were harvested from the cartridge over the course of the 3 months of culture. The number of embryoid bodies harvested did not decrease over time. When these harvests were placed into flask culture a certain population of cells would attach to the plastic while the embryoid bodies remained.

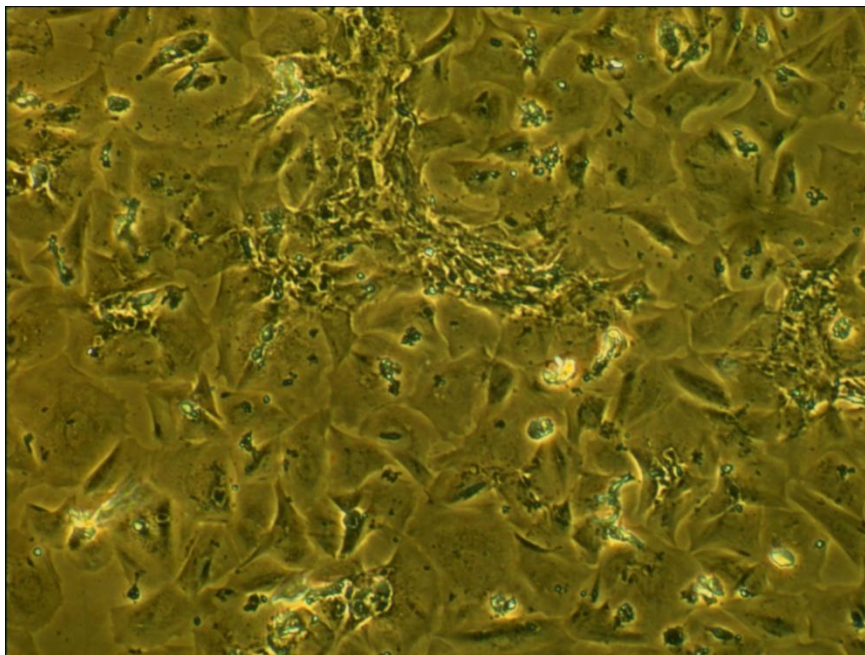


Figure 3 Cells harvested from placenta and cultured in flasks for 3 weeks

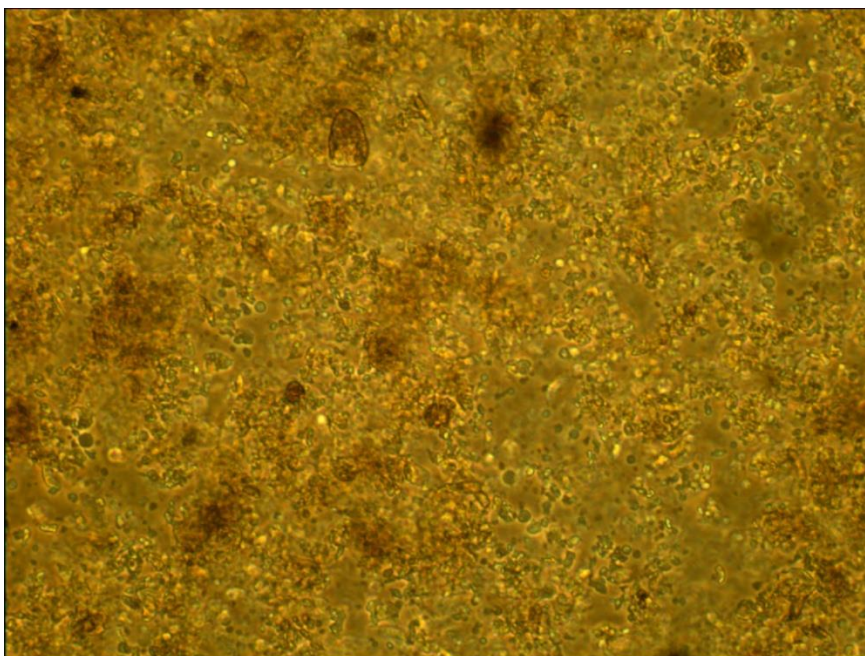


Figure 4 Raw harvest from 5kd MWCO cartridge after 6 weeks of culture. Note clusters of cells that resemble embryoid bodies.

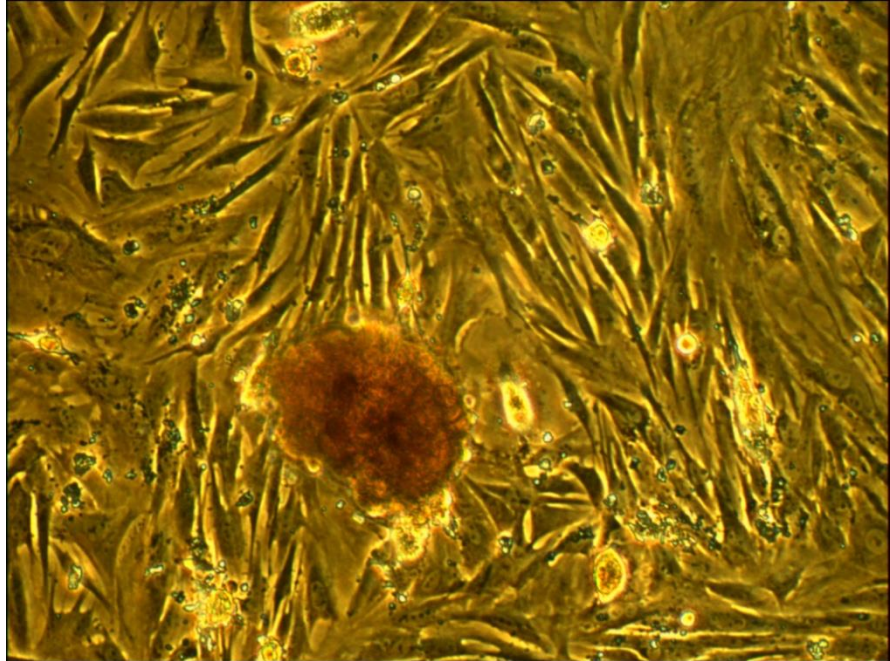


Figure 4 Higher magnification of above.

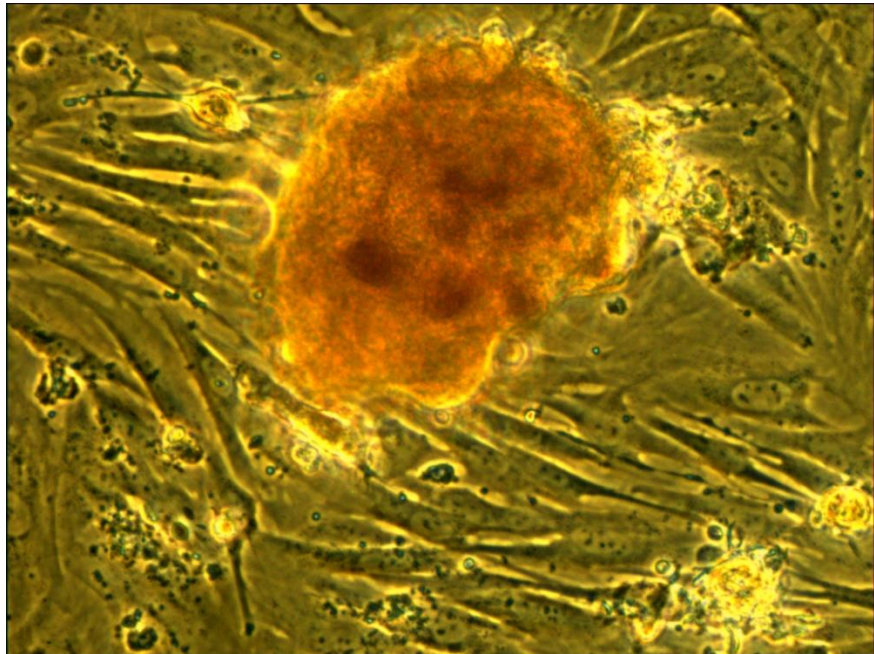


Figure 5 Same as above, different field.

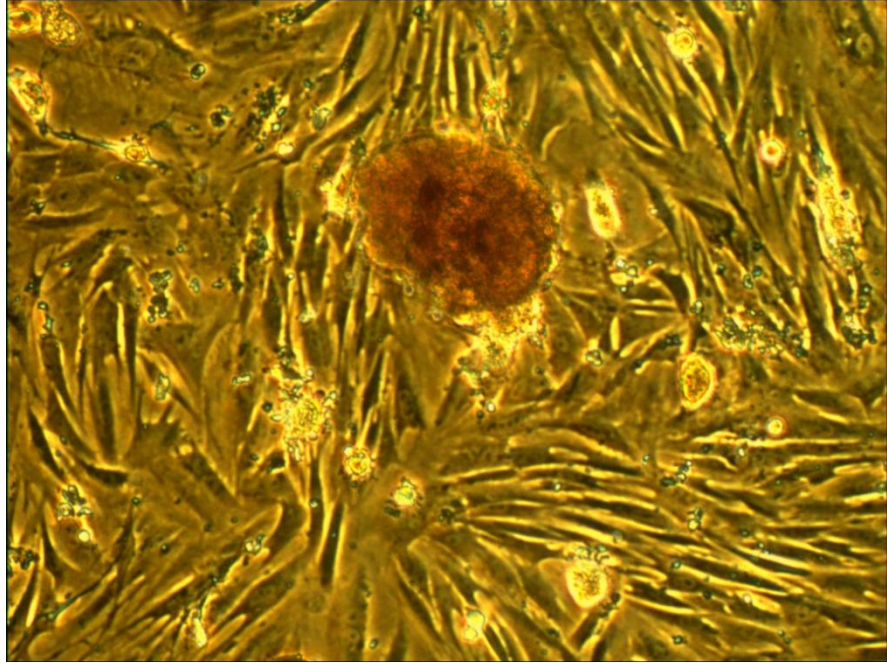


Figure 6 Same as above, different field.

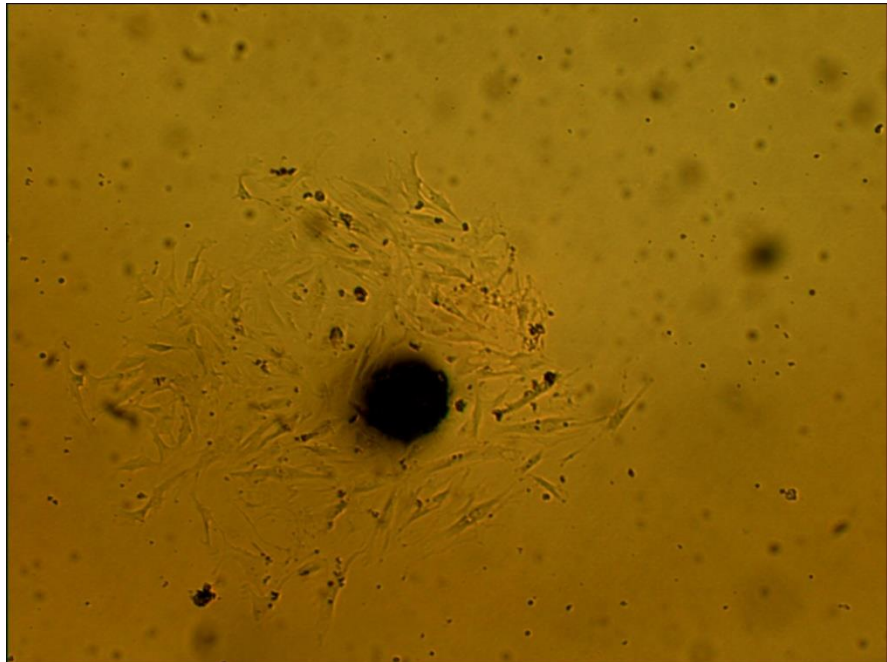


Figure 7 Same as above, different field, lower magnification.

Discussion

Hollow fiber bioreactors offer a unique environment for the culture of cells.

Cells are bound to a porous support much as they are *in vivo*. There is no requirement to split cells. Cells in this perfusion system maintain viability and production-relevant metabolism in a post-confluent manner for extended periods of time, months or longer. The more *in vivo* like growth conditions afforded by hollow fiber bioreactors result in significantly reduced apoptosis (1).

The molecular weight cut off (MWCO) of the fiber can be controlled. Desired products can be retained to significantly higher concentrations and the effects of cytokines can also be controlled.

Extremely high surface area to volume ratio. The small diameter of the fibers (200 μ) generates an extremely high surface area to volume ratio in the range of 100-200cm²/ml of volume. Coupled with the high gross filtration rate of FiberCell® System's polysulfone fibers the exchange of nutrients and waste products is very rapid. Cell densities of 1-2X10⁸/ml or more are achieved, close to *in vivo* tissue-like densities.

The goal of this application note was to evaluate the response of a mixed population of cells derived from the placenta to these unique culture conditions. Previous data had shown a significant increase in glucose uptake rate when the 5kd MWCO cartridge was used as opposed to the 20kd MWCO cartridge. This is likely due to the retention of cytokines within the extra-capillary space facilitating cell-to-cell interactions. The porous nature of the hollow fiber support permitted the culture of primary human cells to be grown in culture for at least three months. During this time a significant number of cells and what appeared to be embryoid bodies (clusters of cells) along with other cell were generated within the cartridge. When these cells were plated a certain portion of this population would attach and display interesting morphology while the embryoid bodies themselves seemed to remain intact. The attaching cells appeared to be associated with the clusters of cells as demonstrated by figure 7.

This data shows that the use of hollow fiber bioreactor cartridges can support the culture of primary human placental derived cells for extended periods of time, at least three months. During this time embryoid bodies/cell clusters are generated and require further characterization. This method can act as a replacement for the separation of adherent from non-adherent cell types performed in flask culture with the advantages being that larger numbers of cells can be processed at one time. Cell to cell interactions, facilitated by the smaller molecular weight cut-off appear to be important.

Surprisingly little work with stem cells and hollow fiber has been reported in the literature. Hollow fiber bioreactors are also an effective means for the generation of conditioned medium and for cellular co-cultivation.

Further work will characterize the cells generated by this system and optimize further the culture conditions. Hollow fiber bioreactors offer a unique environment for the culture of primary cells and particularly with mixed populations of cells.

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