

## SUD INCLINED SETTLING BIOREACTOR FOR GENTLE EXPANSION, CONCENTRATION & HARVEST OF GENE & CELL THERAPIES

Dhinakar S. Kompala, Sudhin Biopharma Co.  
kompala@sudhinbio.com  
Christopher B. Glascock, Sudhin Biopharma  
Braden L. Smith, Sudhin Biopharma  
David A. Aberin, Rockwell Automation

**Key Words:** Single Use Disposable, Inclined Settling Bioreactor, Cell Therapy Manufacturing, Gentle Cell Separation or Harvest, Scalable low-shear bioreactor

Inclined settling technology has been successfully demonstrated in biopharmaceutical manufacturing as a selective cell retention device for achieving high cell densities and viabilities for an extended culture duration (over 3 months, Bayer 1990s) in perfusion bioreactors and at large scales (3000L, Roche, 2013). We have developed a novel compact scalable design in a conical and cylindrical geometry with a smaller footprint and 6x higher inclined settling surface area and demonstrated that these compact settlers can settle smaller yeast cells to be recycled to a high cell density perfusion bioreactor operated over 2 months (Freeman, et al. 2017).

Following the Industry trends into single use technologies, we have fabricated the compact settlers as single use disposable plastic BioSettlers and demonstrated their unique and essential functions of selective cell retention and recycle of live and productive CHO cells back to the bioreactor and continuous removal of dead cells and cell debris from the top of BioSettler in our laboratory. Our collaborators at Pluristyx have demonstrated that the smallest BioSettler (250 ml) can settle all (100%) of iPSC clusters (>100 microns), while removing only the smaller single cells (live or dead) and cell debris via the settler's top effluent.

By installing single use disposable pH and DO fluorescent dye sensors inside the BioSettler and manipulating the mix and flow rates of air, O<sub>2</sub> and CO<sub>2</sub> sparged into the cell culture medium inside the BioSettler, our collaborators at Rockwell Automation have demonstrated feed-back control of pH and DO within the physiological or optimal cell growth range inside the BioSettler in our laboratory as shown in the Figure 1 to the right. As cell culture liquid medium gently circulating inside the BioSettler is now controlled at any desired or optimum cell growth conditions of pH, DO and T, any mammalian cells can be grown inside this novel "Inclined Settling Bioreactor (ISB)". This novel bioreactor combines the desirable features of a gentle air-lift cell culture bioreactor with the selective cell retention features of the inclined settler, combined to a single device. We will present latest data from our current CHO cell culture operated in a perfusion mode to remove smaller dead cells and cell debris selectively from the top of the ISB, while the larger and productive CHO cells are retained to high cell densities and productivities inside the ISB. A second ISB is being fabricated to culture iPSC clusters inside the low-shear bioreactor at Pluristyx, so that the larger iPSC clusters are retained in the ISB, while the spent medium is removed along with any single dead or live cells to be replaced with fresh medium daily or in continuous perfusion mode.

This novel Inclined Settling Bioreactor will be useful for growing not only suspension cells like CHO, iPSCs, but also any attachment dependent cells like MSCs on microcarrier beads and HEK cells on inclined settling cones, while their product AAV vectors can be harvested easily from the top.

