Disposable TubeSpin® Bioreactor 600 offers superior cell culture performance and larger working volume for suspension cultures of mammalian cells.

In 2004, disposable, pre-sterilized and ventilated polypropylene “Bioreactors” called TubeSpin® Bioreactor 50, with a volume of 50 ml, were established as a novel container for suspension cultures of mammalian cells. Work towards this product was executed in a collaboration among the Swiss Federal Institute of Technology in Lausanne (EPFL), the company ExcellGene SA in Monthey, and a leading provider of plastic products for tissue culture and laboratory technology, TPP Techno Plastic Products AG in Trasadingen (all in Switzerland)[(1)]. The TubeSpin® Bioreactor 50 have become a widely accepted addition to the tool box of scientists who wish to perform small scale (5 to 35 ml) suspension cultures with growth performance equivalent to fully controlled bioreactors. The usefulness of the TubeSpin® Bioreactor 50 is based on two advantageous characteristics. When shaken appropriately, these vessels provide to cells the highest oxygen supply observed in a non-instrumented container under conditions of extremely low shear stress. This combination of features allows certain suspension cell lines, for example CHO cells, to grow to densities of 10 mio cells/ml and higher, if the medium is rich enough to support such cell densities. The reactors are applied in CO_2 controlled, humidified shaker incubators which provide efficient liquid mixing and gas exchange from the headspace to the liquid phase. Under appropriate CO_2 concentrations in the incubator, the pH of the cultures can be maintained for the entire cultivation period in a narrow, physiological range. Shaking speeds of 150 to 250 rpm are used depending on the working volume in the TubeSpin® Bioreactor 50. Due to their small footprint, a single large incubator shaker with 4 shaking platforms (Kühner Climo Shaker ISF4-X) allows the simultaneous culture of almost 500 individual containers.

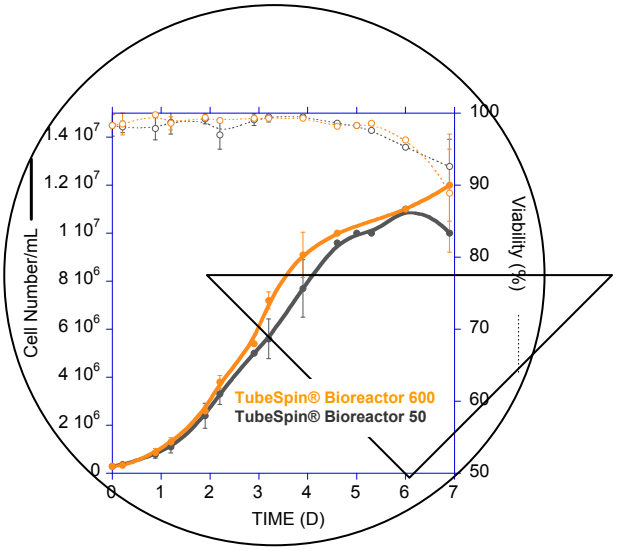
Recently, a new 600 ml vessel, named TubeSpin® Bioreactor 600, has been developed and is being introduced to the market to provide a scale-up option for lab use. This bioreactor can be used with working volumes up to 400 ml. Figure 1 shows results obtained when comparing cultures of CHO cells in TubeSpin® Bioreactor 50 (5 ml cell culture) and the new TubeSpin® Bioreactor 600 with a working volume of 100 ml.

[1] Additional support and technical advice came from the company Kühner AG, Birsfelden, Switzerland provider of shakers to industry for six decades.

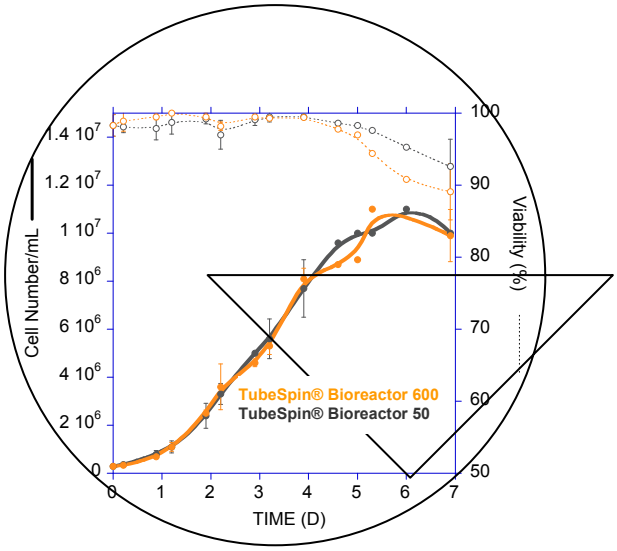
Fig. 1: TubeSpin® Bioreactor 50 versus TubeSpin® Bioreactor 600 - culture of CHO cells.

Cell culture over 7 days in TubeSpin® Bioreactor 50 bioreactors (5 ml) and in TubeSpin® Bioreactor 600. Both bioreactors were shaken at 180 rpm in a Kühner incubator shaker supplied with 5 % CO_2 and 80 % or higher humidity. The TubeSpin® Bioreactor 600 culture had a working volume of 100 ml (a) or 400 ml (b).

a)



b)



The comparison demonstrated equivalent culture performance over a 10 to 40 fold volume increase, and verified the capacity to grow CHO cells to high density, up to 1 x 10^7 cells/ml, in an orbital shaken 600 ml disposable plastic vessel. To our knowledge this is the highest cell density ever observed in a non-instrumented vessel using a simple batch cultivation mode, i.e. with no feed additions. The above mentioned shaker can be provided with 1 to 5 platforms with up to 12 TubeSpin® Bioreactor 600 holders per platform, thus allowing a total of 60 parallel cell cultures in volumes from 100 to 500 ml.

In preliminary engineering studies performed at the Swiss Federal Institute of Technology Lausanne, Switzerland (EPFL), the gas transfer rate in the new TubeSpin® Bioreactor 600 was studied. The gas transfer occurs passively through the sterile membrane barrier in the cap of the reactor. k_La values were determined at shaking speeds between 130 and 260 rpm for a reactor with a working volume of 500 ml (Fig. 2). First, the oxygen levels were reduced to 0 % by sparging nitrogen into the vessel, and then the kinetics for establishing O_2 saturation (relative to air) were determined and the gas-transfer coefficient k_La was derived.

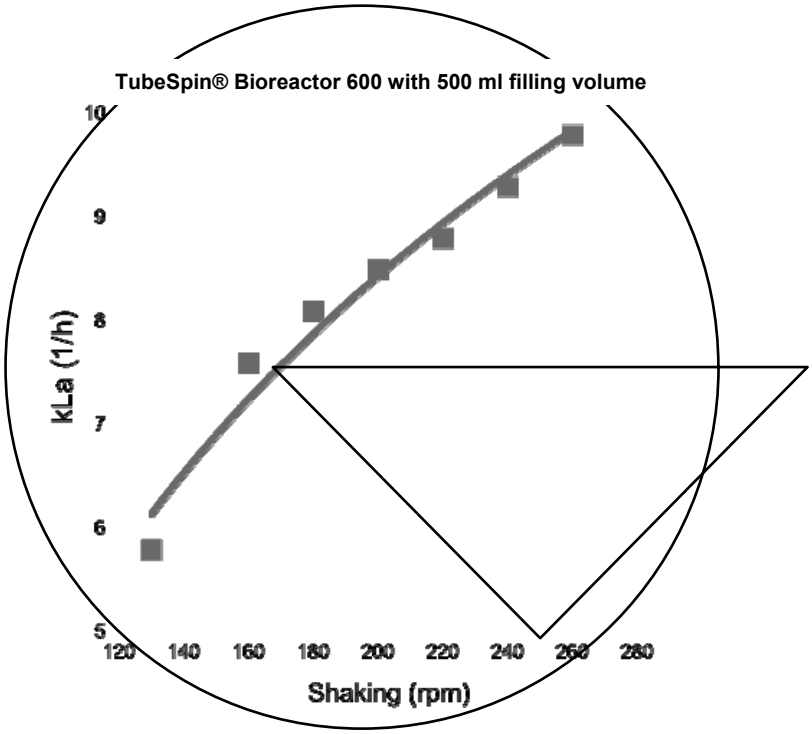


Fig. 2: Gas-transfer rate determination in a TubeSpin® Bioreactor 600 using the gas-out/gas-in method.

The k_La values (including the expected gas transfer resistance from the sterile membrane barrier and the restriction through the holes in the cap) provide the engineering basis for the observed cell culture densities of up to 1 x 10^7 c/ml. Sufficient air can be exchanged between head space and liquid phase to provide cells in suspension with the necessary oxygen, thus avoiding limitations on the metabolic activity of highly proliferative cells.

Scientific and engineering contributions of the following persons are acknowledged: Cedric Bürki, Gilles Broccard, Maria De Jesus, all ExcellGene SA, Monthey, as well as Stephanie Tissot, Noushin Hadadin and Florian M. Wurm at the Swiss Federal Institute of Technology, Lausanne (EPFL).