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SuperSpinner D 1000: a disposable bioreactor for efficient lab-scale cultivation of animal cells

Sufficient oxygen supply is essential for proper function of all energy-consuming processes during the cell cycle. Oxygen limitation results in slow growth and production kinetics. Effective aeration during cell cultivation is one requirement to achieve high cell densities and high product concentrations. The SuperSpinner D 1000 is a disposable bioreactor that ensures optimal oxygen supply and high productivity during the cultivation of animal cells in a scale of 1 liter.

In controlled cultivation systems, such as common stirred tank bioreactors, an optimal oxygen supply is state of the art. Special control cascades via adaptation of agitation, gassing rate and/or oxygen saturation are used to circumvent oxygen limitations. However, this control is not available in common lab cell-cultivation devices, which mainly consist of easy-to-use and low-cost equipment. The majority of these devices are surface aerated, and are thus limited by the horizontal liquid surface. This often results in oxygen limitation; optimal cell density and product concentration cannot be achieved by using traditional spinner flasks, for example.

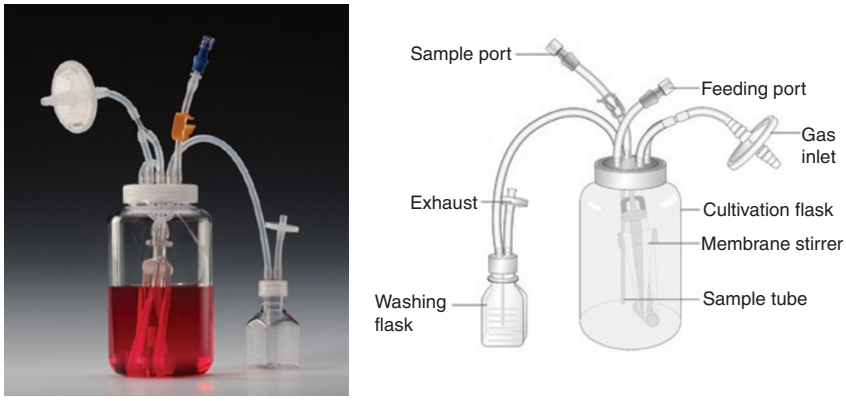

Figure 1 | SuperSpinner D 1000: product picture and schematic.

The SuperSpinner D 1000 is a disposable stand-alone cultivation unit, which was developed for efficient cell cultivation by ensuring an optimal oxygen supply without using sophisticated equipment and high stirring rates. It is easy to use and enables cost-efficient, laboratory-scale cell cultivation with a recommended maximum working volume of 1 liter.

The key feature of the SuperSpinner D 1000 is an integrated membrane aeration and agitation system, which allows for optimal gas transfer during cell cultivation (**Fig. 1**). A hollow-fiber membrane is wound around a stirrer bar, the latter containing an iron core at the tip. Agitation is achieved by a magnetic drive unit. A membrane pump complements the setup; it feeds ambient air through a sterile filter into the hollow-fiber membrane. Under working conditions oxygen and carbon dioxide diffuse from the hollow-fiber membrane into the cell suspension. The multiple windings of the hollow fiber represent an enormous increase in the (active) aeration surface. Thus, oxygen is not limiting to cell growth, which results in higher productivity compared to that in traditional spinner flasks. The assembly can be placed in an incubator (CO_2 incubator, for instance), if special gases are necessary.

The SuperSpinner D 1000 is supplied completely preassembled. A special sampling system enables sterile sampling. Furthermore, feeding is possible at any time of the cultivation by using the spare port, which is closed with a Luer-lock connector. The cultivation flask itself (total volume, 1 liter) is compatible with standard rotors, that is, the cell suspension can be centrifuged in the cultivation flask. If the contents of the SuperSpinner D 1000 is intended to be used as seed for a larger bioreactor, the cultivation broth can be transferred directly from the SuperSpinner D 1000—for example, by pumping. For this purpose transfer tubing can be affixed to the Luer-lock connector of the sample port.

We evaluated the SuperSpinner D 1000 for the cultivation of different cell lines. Here we describe the cultivation of *Spodoptera*

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APPLICATION NOTES

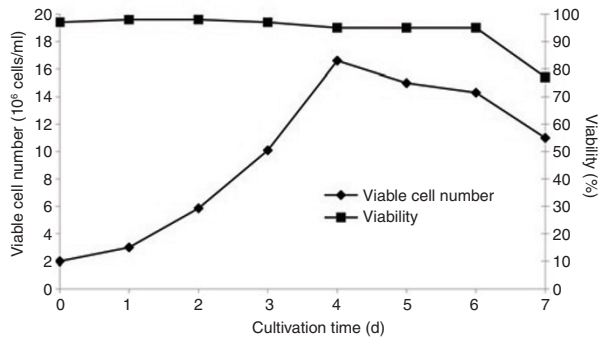

Figure 2 | Batch cultivation of Sf9 cells in Sf-900 III medium at 27 °C, 75 r.p.m. and 0.1 v.v.m. Samples were collected daily and analyzed for viable cell number and viability.

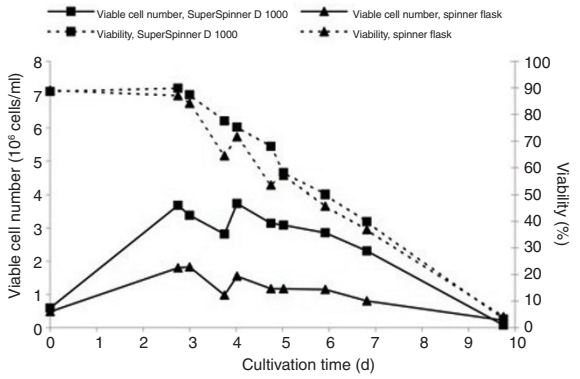

Figure 3 | Batch cultivation of CHO cells in proCHO5 medium at 37 °C, 60 r.p.m., with aeration via a membrane pump and at 5% CO_2 saturation in SuperSpinner D 1000 and a traditional spinner flask. Samples were collected daily and analyzed for viable cell number and viability.

frugiperda Sf9 cells and Chinese hamster ovary (CHO) cells in a SuperSpinner D 1000.

Cultivation of *Spodoptera frugiperda* Sf9 cells

Sf9 cultivation studies were carried out by R. Eibl (Zurich University of Applied Sciences). Eibl and colleagues grew Sf9 cells (nontransfected) in Sf-900 III medium (serum free, 1-liter working volume) at 27 °C in an incubator, with the stirring rate of the magnetic drive unit adjusted to 75 r.p.m. The cells were aerated with 0.1 v.v.m. (gas volume/liquid volume/minute; gassing rate was adjusted by using the rotameter system of the incubator). The starting cell density was 2×10^6 viable cells/ml. To determine the (viable) cell number and the viability, they collected the samples once a day without moving the bioreactor by connecting a sterile syringe to the clave adapter.

A maximum viable cell number of 1.66×10^7 cells/ml was achieved at day 4 after inoculation (**Fig. 2**). Cultivation in a traditional spinner flask under the same conditions resulted in a cell density of 4–5 $\times 10^6$ viable cells/ml (personal communication; R. Eibl).

Cultivation of CHO cells

We grew CHO cells in proCHO5 medium (0.8 liter working volume) at 37 °C in a CO_2 incubator with a CO_2 saturation of 5%. The starting cell density was 1×10^6 viable cells/ml, and stirrer speed was 60 r.p.m. A membrane air pump was installed as an aeration system. We collected samples as for Sf9 cells.

The cultivation of CHO cells in the SuperSpinner D 1000 resulted in a maximum viable cell number of ~4×10^6 cells/ml after 3 days of cultivation, whereas the maximum cell density attained in a traditional spinner flask cultivation was <2×10^6 viable cells/ml (**Fig. 3**).

Conclusion

Cultivation of Sf9 cells in the SuperSpinner D 1000 resulted in a maximum viable cell number that was >3 times higher than that of cells grown in a traditional spinner flask operating with surface aeration (personal communication; R. Eibl). When cultivating CHO cells in the SuperSpinner D 1000, a twofold increase in the viable cell number could be observed compared to that in a classic spinner flask.

In other studies the SuperSpinner D 1000 was used to cultivate murine hybridoma cells. In these studies, a maximum cell density of 3.16×10^6 viable cells/ml was achieved, which is ~2.25 times higher than in traditional spinner flasks (personal communication; C. Schwiebert, InVivo BioTech Services GmbH).

These data show that the SuperSpinner D 1000 delivers higher cell densities than a traditional spinner flask and thus provides a simple solution for efficient lab-scale cultivation of different cell cultures.

ACKNOWLEDGMENTS

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