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# A VERSATILE DISPOSABLE CULTURE SYSTEM FOR HIGH THROUGHPUT SCREENING OF PROCESS PARAMETERS AND PRODUCTION CELL LINES

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## 1. INTRODUCTION

There is no doubt that fully equipped and controlled bioreactors are the only valid system for industrial production of large amounts of proteins with classical cell culture technology. However, valuable information for bioprocesses can be obtained much faster and easier using alternative cultivation systems. For attached cells, different disposable culture plates or flasks satisfy most of these needs. Since fewer choices are available for suspension cultures, we wanted to develop a reliable and versatile system that fits routine applications in our laboratory. To support high cell densities, the system needs to provide good mixing of the medium and efficient exchange rates for oxygen and carbon dioxide.

We used 50 ml centrifugation tubes with ventilation caps (Techno Plastic Products, Trasadingen, Switzerland) having five holes with different diameters above a gas permeable sterile filter (Fig. 1, right panels). The holes can be closed easily by covering them with adhesive tape to regulate gas exchange rates between the inside and the outside of the tube. Here we present the first results demonstrating the flexibility of these tubes for growing suspension cells to high densities.

## 2. RESULTS

The 50 ml centrifugation tube with a ventilation cap is an open system with tuneable permeability for gases such as $O_2$ and $CO_2$. The permeability or mass transfer coefficient of the filter caps is not only relevant for $O_2$ and $CO_2$ exchange but also defines the evaporation rate, an important parameter for small culture volumes since the humidity within the tube is approximately 100%, while outside the tube it is typically 30% - 85%. Measuring the evaporation rate is straightforward and yields valid information about the mass transfer coefficient of the caps. Tubes containing 10 ml of DMEM/F12 medium were shaken at 37°C for 5 days (200 rpm with a shaking diameter of 5 cm, humidity about 35%). One or more holes of each cap was left open during the course of the experiment. For each tube the loss of medium due to evaporation is plotted in Fig. 1.

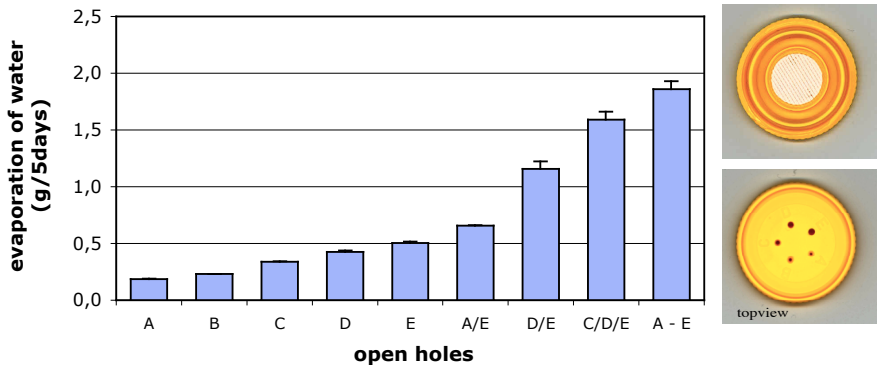


***Figure 1**: Permeability of the filter caps. Left: evaporation during 5 days at 200 rpm in a 37° room without control of humidity. The five holes were opened individually or in various combinations (n=2, except for B where n=8). Right: Interior of cap with gas permeable sterile filter (top) and exterior of cap with five holes of different diameter (bottom).*

Evaporation from the tubes correlated with the diameter of the open hole and was additive if several holes were left open. When all the holes were closed there was no evaporation (data not shown). The results demonstrated that the evaporation can be precisely modulated over a wide range. The maximum loss of water (2 ml per 5 days) exceeds the actual needs for animal cell cultures. This is twice the amount we have seen for a 1L Bellco spinner with a 300 ml culture volume and one cap opened.

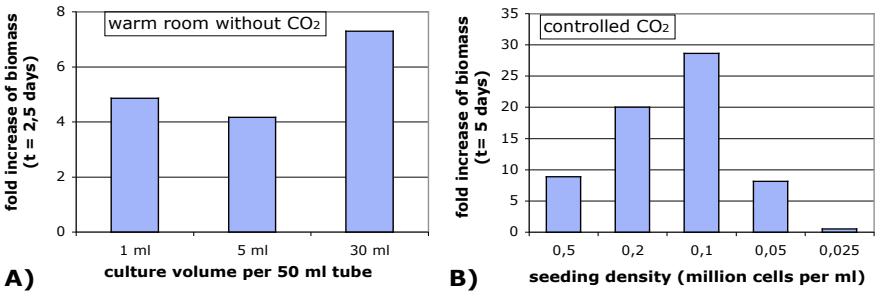


***Figure 2**: Growth of CHO DG 44 shaken at 180 rpm in serum free medium. **A)** Tested culture volumes: 1 ml (tubes were preincubated for 1 hour at 5% $CO_2$ and then closed), 5 ml (hole A open) and 30 ml (hole E open). **B)** 5 ml cell suspension of 25'000 to 500'000 cells per ml were incubated for 5 days in a $CO_2$ incubator (hole E open).*

Based on these results and previous studies addressing the impact of carbon dioxide exchange on the medium pH, a variety of growth conditions in the ventilated centrifuge tubes were tested using CHO DG44 cells. In one experiment the culture volume ranged from 1 ml to 30 ml and the initial cell density was 500,000 cells/ml. The cultures were incubated for 2.5 without feeding, pH adjustments, or changes in filter permeability. Cells grew in volumes of 1 – 30 ml under normal atmosphere (Fig. 2A). In a second experiment the culture volume was

maintained at 5 ml and the initial seeding density was varied from 25,000 to 500,000 cells/ml. The cultures were maintained for 5 days in a shaker with $CO_2$ control. In all cases hole E of the cap was left open. Only at an initial density of 25'000 cells/ml did the cells fail to grow, while for the other cases an increase in biomass up to 30-fold was obtained (Fig. 2B). Cell viabilities were over 90% even for the highest seeding density (data not shown). For this culture the maximum cell density was expected much earlier than day 5. Surprisingly, no aggregate formation or cell settling was observed in this culture.

The effect of $CO_2$ control on cell growth in 50 ml tubes was investigated with HEK293 cells starting at 350,000 cells/ml in a volume of 5 ml. Under a controlled $CO_2$ atmosphere (5%), different permeabilities of the caps worked well (Fig. 3). If $CO_2$ was not present in the atmosphere, the permeability of the caps was crucial for cell growth; increasing the permeability had negative effects (Fig. 3). The tubes that were opened at day 0 did not support cell growth due to the basic pH of the bicarbonate buffered medium. Surprisingly, there were no pH differences at day 4 for all the other cases.

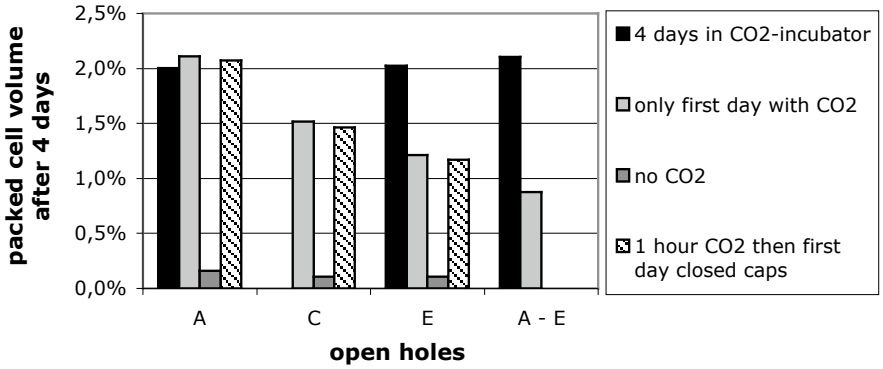


***Figure 3**: The effect of the permeability of the filter caps on the growth of HEK 293 cells under serum free conditions (a packed cell volume of 2% corresponds to a density of 4.5 million cells/ml).*

## 3. CONCLUSION

The first results from cell growth experiments in the ventilated centrifuge tubes are promising. The geometrically defined caps avoid physical differences between individual tubes. The need for only a few ml of culture to launch experiments that can run for up to two weeks under a $CO_2$ and humidity controlled atmosphere make these tubes a unique system for growth of suspension cells. Due to their ease of handling, these tubes represent a valuable tool for high throughput experiments for screening and for process optimization. The system is also suitable for the routine passaging of cells. With tuneable filter caps the system can be adapted to a large variety of culture conditions ranging from 1 ml to 30 ml and from 0.1 – 6 million cells per ml.