Rapid, serum-free, high-yield antibody production in Nunc PETG Roller Bottles

Abstract

Monoclonal and polyclonal antibodies have been an essential tool for research for well over two decades. more recently, antibody-based therapy has emerged as an important approach for the treatment of a number of diseases. In this report, we describe a comprehensive system for antibody production using Thermo Scientific™ Nunc™ PETG Roller Bottles. This serum-free system (TIB-105) allowed the expansion of a hybridoma cell line growing in a single T-flask to a maximum cell density of ~1.2 x 10^6 cells/mL in 8 days, with a peak antibody concentration of 230 µg/mL by 17 days, yielding about 184 mg per 800 mL roller bottle. By harvesting cells at peak viable cell count and subpassaging 1 roller bottle to 10 additional roller bottles, virtually unlimited scalability would be possible with no change in the peak cell density or peak antibody concentration in subsequent passages. These data suggest that by the 4th passage, the system could produce more than 18 g of antibody from a single T-80 flask within 21–24 days.

Introduction

The demand for antibodies in various applications has rapidly increased the need for improved systems for scalable production. Traditional bioreactors often do not allow production to be scaled as needed. Due to the FDA's growing concerns regarding the use of animal serum, combined with the need for downstream antibody purification, serum-free media for hybridoma culture have become increasingly popular. Here we introduce a rapid, flexible, and high-yield approach both hybridoma expansion and for antibody production using a serum-free medium in Nunc PETG Roller Bottles. With this system, hybridomas from a single T-80 flask can be expanded to 1 x 10^{11} cells in as few as 18 days and produce as much as 18 g of antibody in the serum-free medium within 21—24 days.

Materials and methods

Roller bottles: Nunc PETG Roller Bottle, Cat. No. 1060-20

Hybridoma: Hybridoma TIB-105 cells (ATCC) producing monoclonal IgG2a anti–Lyt-2 antibody were adapted to serum-free medium, which is composed of Hyclone™ SFM4Mab (GE Healthcare Life Sciences), 20 mm HEPES, and 1X antibiotic/antimycotic solution.

Cell growth and antibody production: A two-step approach for cell growth from a Thermo Scientific™ Nunc™ T-80 flask to a Nunc PETG roller bottle was designed. Hybridoma cells from one T-80 flask (3 x 10^7 cells in ~30 mL of medium) were first seeded into 270 mL of serum-free medium in a roller bottle (1:10 dilution). After 4 days of incubation, an additional 500 mL of fresh medium was added to the same Nunc PETG roller bottle (passage 1, total 800 mL/roller bottle).

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Thermo Scientific™ Nalgene™ sterile, single-use shaker flasks and Nunc T-80 flasks were used for comparison at the same cell seeding density and the same two-step method employed. Hybridomas (passage 1) were counted after 3, 4, 6, 8, 11, 14, and 17 days of incubation, and samples of the medium were collected for antibody quantification after 8, 11, 14, and 17 days of incubation.

Passage of hybridoma cells in Nunc PETG roller bottles: To expand cells for larger quantities of antibodies, hybridoma cells were subpassaged in Nunc PETG roller bottles for up to 3 passages. On day 8, the viable cell density peaked, and 8 x 10^7 viable cells (~80 mL) from the first roller bottle (passage 1) were seeded into 720 mL of fresh medium (total 800 mL/roller bottle) in Nunc PETG Roller Bottles (passage 2). When the viable cell density (passage 2) peaked after 5 days of incubation, cells were again subpassaged. For comparison, cells from roller bottles were also passaged into Thermo Scientific™ Nalgene™ Erlenmeyer Flasks (~18 mL passaged into 180 mL) and Nunc T-80 flasks (~3 mL passaged into 30 mL). The hybridoma cells were counted after 5 days of incubation, and samples of medium were collected after 8 and 11 days of incubation for passages 2 and 3.

Cell culture: Cells in Nunc PETG roller bottles were incubated at 37°C in a roller bottle incubator, with a rolling speed of 60 revolutions per hour. Cells in Nalgene Erlenmeyer Flasks were incubated in a Thermo Scientific™ shaking incubator at 100 rpm, and cells in Nunc T-80 flasks in a humidity-controlled CO_2 incubator.

Antibody purification and measurement: The antibody in samples of medium was purified and measured by absorbance at 280 nm with reference to a BSA standard curve.

Results and discussion

Serum-free incubation

To determine antibody purity, TIB-105 cells were incubated in serum-free medium, to avoid contamination by antibodies and other serum proteins in the final purified product. Antibody purity was confirmed by SDS-PAGE under denaturing conditions (Figure 1).

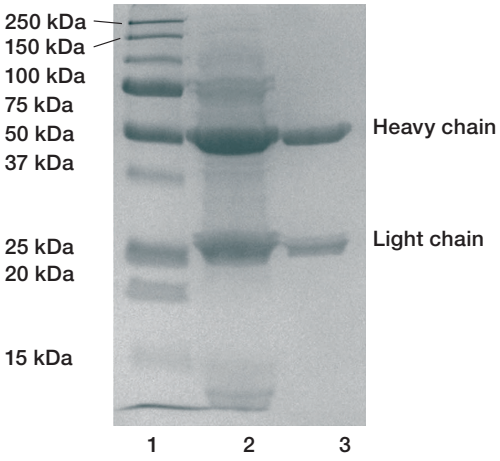

Figure 1. SDS-PAGE of antibody before and after purification from serum-free medium. Antibody was purified from samples of the culture medium and quantified by absorbance at 280 nm. Lane 1: protein molecular weight markers; lane 2: unpurified serum-free medium; lane 3: purified antibody.

Seeding in a Nunc PETG roller bottle from a single Nunc T-80 flask

For TIB-105 cells, the optimal seeding density for passaging is 10^5 viable cells/mL or higher upon (~1:10 dilution). The volume of culture grown in a single T-80 flask can be used to seed a total volume of 300 mL. The 300 mL was seeded (with the 30 mL from the T-80 flask), within the confines of the roller bottle and brought to capacity (800 mL) 4 days later by addition of 500 mL serum-free medium.

Alternatively, three Nunc T-80 flasks could be used to directly inoculate 710 mL of serum-free medium. The optimal time for cell harvesting is day 8, when the cell density peaks. The antibody concentration peaks after day 11 and is maintained at this high level up to at least day 17. The total antibody produced per Nunc PETG roller bottle was 163.2–184.3 mg after 11–17 days of incubation (Figure 2).

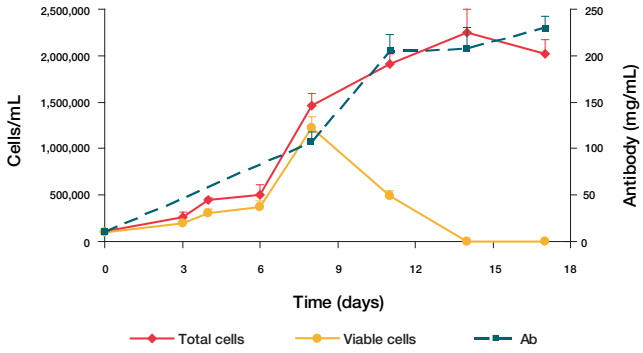

Figure 2. Time course of hybridoma TIB-105 culture growth and antibody production in serum-free medium. 3 x 10^7 cells from T-80 flasks (~30 mL) were seeded into 270 mL of serum-free medium (total 300 mL/roller bottle). After 4 days of incubation, an additional 500 mL of medium was added into the same roller bottle (total 800 mL/roller bottle).

Seeding in a Nunc PETG roller bottle from Nunc PETG roller bottles

Hybridoma cells were subpassaged into additional Nunc PETG roller bottles, Nalgene Erlenmeyer flasks, and Nunc T-80 flasks by 1:10 dilution. The data shows that hybridoma cells from Nunc PETG roller bottles continue to grow well in all three culture vessel types (Figure 3). Cultures grown in Nunc PETG roller bottles can be used as the source of cells for future expansion.

Using Nunc PETG roller bottles to rapidly produce large quantities of antibodies

Since hybridoma cells can be expanded from 1 roller bottle to 10 roller bottles in 5 days of incubation, rapid, and scalable expansion of hybridoma cells can be employed to produce large quantities of antibodies. For example, after 3 passages, 100 roller bottles can be seeded, producing up to 10^{11} cells in 18 days and/or >18 g of antibodies in as little as 21 days (Figure 4).

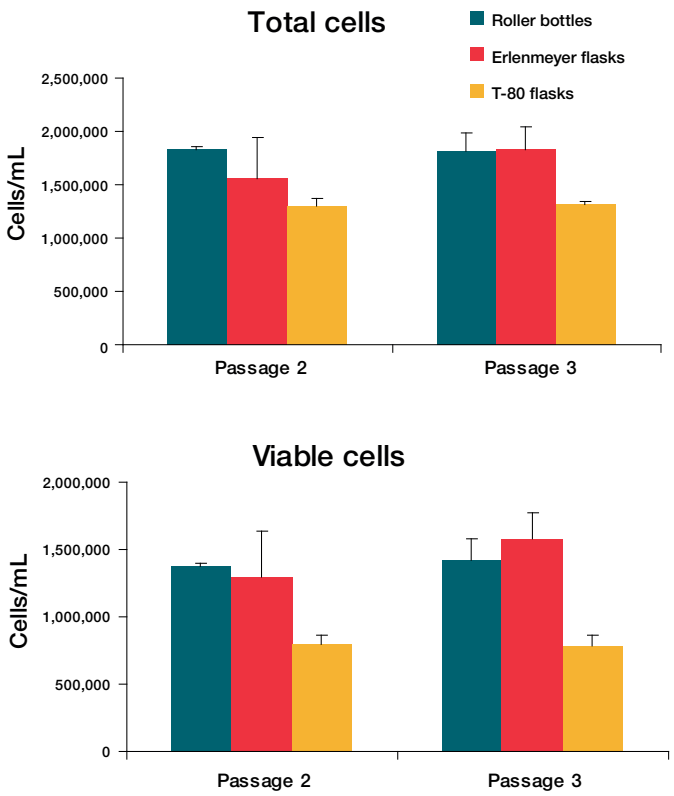

Figure 3. Proliferation of hybridoma subpassaged from Nunc PETG roller bottles. Hybridoma were seeded into three different culture vessel types. After 5 days of incubation, total and viable cells were counted with trypan blue.

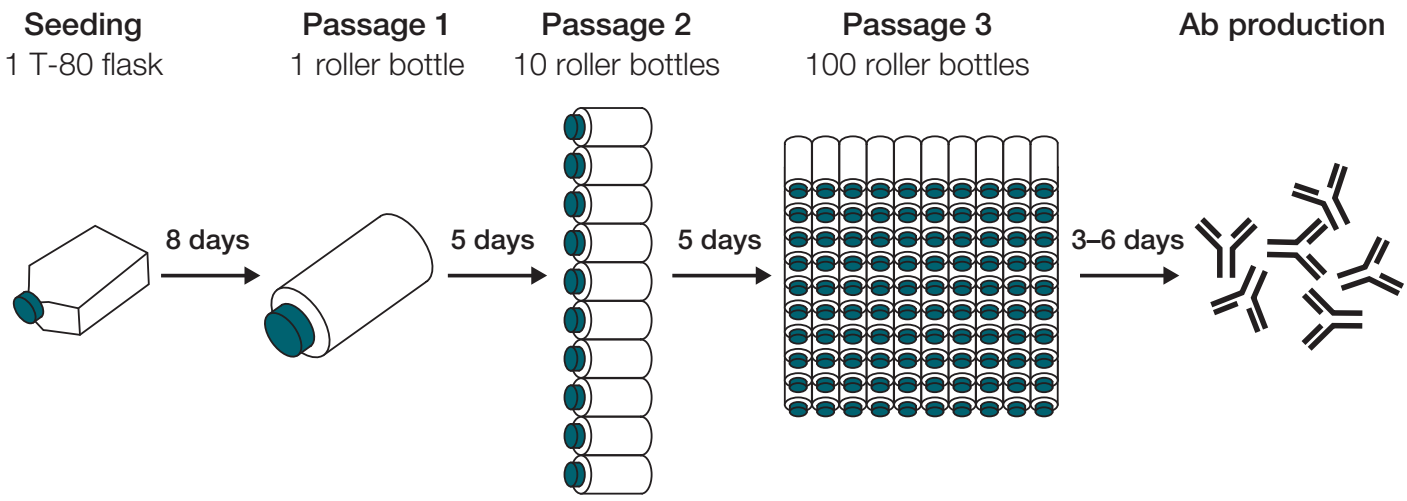

Figure 4. Cell expansion and antibody production from one T-80 flask to 100 roller bottles.

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Hybridomas subpassaged from Nunc PETG roller bottles continue to produce high yields of antibodies (Figure 5).

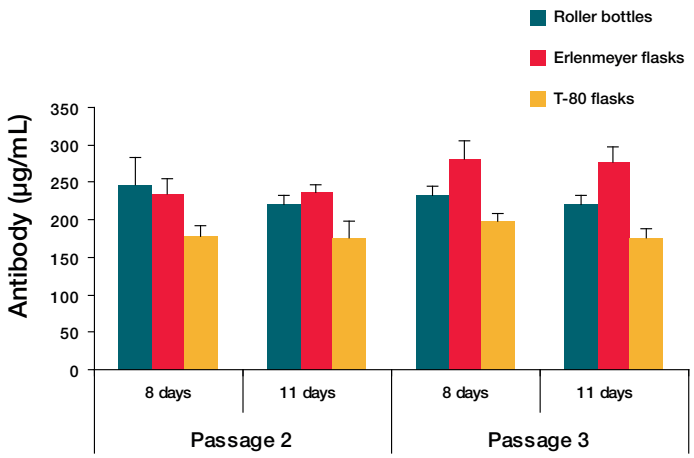

Figure 5. Antibody production by hybridoma in Nunc PETG roller bottles. Hybridoma were subpassaged from Nunc PETG roller bottles into three different culture vessel types. Antibody was purified and quantified by absorbance at 280 nm.

Conclusions

- Hybridomas from one Nunc T-80 flask can be expanded using Nunc PETG roller bottles, to >1 x 10^{11} viable cells within 18 days (Figure 4).
- Hybridoma cells from one Nunc T-80 flask can be expanded to produce >18 g of antibody in roller bottles within 21–24 days.
- The optimal time for harvesting cells for further expansion occurs prior to optimal antibody production.
- Viable hybridoma cells from Nunc PETG roller bottles can be used for future cell expansion or antibody production in other culture vessel types.

References

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