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Development of technology for culturing a cell line producing a single-domain antibody fused with the Fc fragment of human IgG1

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Abstract

Objectives. To develop an effective technology for the cultivation of Chinese hamster ovary (CHO) cells stably producing GamP2C5 antibody which is a component I of the GamCoviMab candidate drug for emergency prevention and therapy of infection caused by SARS-CoV-2 virus; to select optimal cultivation parameters and to scale this technology in production.

Methods. The study was performed on CHO GamP2C5 (clone 78) cell culture, producing a single-domain antibody fused to the Fc fragment of human IgG1 GamP2C5. Different culture media and supplements were used. Cells were cultured in Erlenmeyer flasks, Biostat® RM 20 wave-mixed bioreactor, Ambr® 250 mini bioreactors, STR 200 stirred-tank bioreactor.

Results. Using molecular-genetic and biotechnological methods, a stable clone producer of CHO GamP2C5 antibody, clone 78, was obtained. Then a technique was worked out for the cultivation of the obtained clone producer on different culture media. The most suitable cultivation regimes, culture media, and optimal supplements were selected. This technology was tested in laboratory conditions in a 10-L reactor, and then successfully scaled up for production at the *MedGamal* Branch of the Gamaleya National Research Center for Epidemiology and Microbiology.

Conclusions. This study demonstrates the fundamental feasibility of developing and scaling up a culture technology, in order to produce a drug based on a modified single-domain antibody with virus neutralizing activity against different strains of SARS-CoV-2 virus.

Keywords

monoclonal antibodies, single-domain antibodies, heavy chain antibodies, cultivation, CHO cells, bioprocess scaling

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НАУЧНАЯ СТАТЬЯ

Разработка технологии культивирования клеточной линии, продуцирующей однодоменное антитело, слитое с Fc-фрагментом IgG1 человека

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Аннотация

Цели. Разработать эффективную технологию культивирования клеток яичников китайского хомячка (CHO), стабильно продуцирующих антитело GamP2C5, которое является компонентом I кандидатного препарата ГамКовиМаб для экстренной профилактики и терапии инфекции, вызванной вирусом SARS-CoV-2; подобрать оптимальные параметры культивирования и масштабировать данную технологию на производстве.

Методы. Исследование проводилось на культуре клеток CHO GamP2C5 (клон 78) продуцирующей однодоменное антитело, слитое с Fc-фрагментом IgG1 человека GamP2C5; были использованы различные среды для культивирования и питательные добавки. Культивирование клеток проходило в колбах Эрленмейера, биореакторе с волновым типом перемешивания Biostat® RM 20 basic, минибиореакторах Ambr® 250, биореакторе с осевым типом перемешивания STR 200.

Результаты. При помощи молекулярно-генетических и биотехнологических методов был получен стабильный клон-продуцент антитела CHO GamP2C5 (клон 78), и отработана методика культивирования полученного клона-продуцента на различных питательных средах. Были выбраны наиболее подходящие режимы культивирования, питательная среда и оптимальные подпитки. Данная технология была отработана в лабораторных условиях в 10-литровом реакторе и успешно масштабирована на производстве в филиале «Медгамал» Национального исследовательского центра эпидемиологии и микробиологии им. Н.Ф. Гамалеи.

Выводы. В данном исследовании показана принципиальная возможность разработки и масштабирования технологии культивирования для получения препарата на основе модифицированного однодоменного антитела с вируснейтрализующей активностью против различных штаммов вируса SARS-CoV-2.

Ключевые слова

моноклональные антитела, однодоменные антитела, тяжелоцепочечные антитела, культивирование, клетки CHO, масштабирование биопроцесса

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INTRODUCTION

Following the development of hybridoma technology in the mid-1970s, monoclonal antibodies [1] began to be explored as potential therapeutics for a variety of diseases [2]. Over the last decade, several dozen antibody-based drugs (Adalimumab, Pembrolizumab, Nivolumab) have been developed: some are among the top-selling drugs ranking worldwide [3]. Monoclonal antibodies are used to treat a wide range of human diseases, including various types of cancer [4]. Furthermore, therapeutic antibodies are effective in the therapy of multiple sclerosis [5], Crohn's disease [6], rheumatoid arthritis [7], and bacterial and viral infections [8].

The COVID-19 pandemic had a tremendous impact on drug and vaccine discovery and the development of strategies designed to control infectious diseases. Monoclonal antibodies represent the largest and fastest growing class of pharmaceutical compounds to have shown therapeutic potential in the treatment of viral infections, including against SARS-CoV-2 virus.

In recent years, the number of preparations based on monoclonal antibodies against viral infections, especially chronic infections [9], has increased markedly. Monoclonal antibodies can be used to suppress the spread of infection through direct viral neutralizing activity [10]. To date, several antiviral drugs with a high level of efficacy have been approved. Among them, Casirivimab/imdevimab (REGEN-COV) is the first monoclonal antibody-based drug which can be used for both treatment and emergency prevention of COVID-19 [11].

One particular case of monoclonal antibodies are the heavy chain antibodies of the camelid family. They are devoid of light chains and the variable fragments are represented by a single heavy chain domain. Furthermore, the variable fragments of the heavy chain can be used independently. Such modifications are called nanobodies, also known as single-domain antibodies or VHH [12].

Single-domain antibodies are a relatively new class of drugs. They were discovered by chance while analyzing camel serum. Due to their structure and properties, nanoantibodies are able to effectively bind to antigen epitopes which are difficult to reach for classical antibodies. They also prevent interaction of receptors with ligands, or deliver substances that perform various functions to target cells. At the same time, the disadvantages of nanoantibodies are associated with their rapid excretion by kidneys and a lack of an independent effector function due to the absence of Fc fragment. Many studies demonstrate the successful use of single-domain antibodies and their modifications for therapy and prevention of various infectious diseases of viral and non-viral etiology [13–18]. In order to improve the pharmacokinetic and effector properties of nanoantibodies, as well as to increase their avidity due to the dimerization of the molecule, modification with the Fc fragment of human IgG is used [13, 15, 17].

A single-domain antibody fused to the Fc fragment of human immunoglobulin class, which has a broad spectrum of viral neutralizing activity against SARS-CoV-2 virus and is component I of the GamCoviMab candidate drug for therapy of COVID-19 infection, was developed in the Gamaleya National Research Center for Epidemiology and Microbiology of the Ministry of Health of the Russian Federation. The development of scalable technology for cell culture and chromatographic purification of the target antibody was beneficial to the production of this drug. This article describes our results relating to the development of a strategy to select optimal parameters for culturing processes, in order to produce a single-domain antibody fused to the Fc fragment of human immunoglobulin G class. Data is also presented on *in vitro* scale-up in a 10 L wave-mixed bioreactor and scale-up at GMP production in a 200 L stirred-tank bioreactor.

MATERIALS AND METHODS

Materials

Culture of Chinese hamster ovary (CHO) cells GamP2C5 (clone 78) producing a single-domain antibody fused to Fc fragment of human IgG1 GamP2C5 was obtained in the laboratory of immuno-biotechnology of the Gamaleya National Research Center for Epidemiology and Microbiology on the basis of CHO-K1 cells (Collection of Cultures and Tissues of the Gamaleya National Research Center for Epidemiology and Microbiology).

Cultivation media: ActiPro™ (*Cytiva*, USA), Fujifilm BalanCD® CHO Growth A (*Irvine Scientific*, USA), Cosmos (*Flora Bio*, Turkey), SFM4CHO (*Cytiva*, USA), Dynamis™ AGT™ (*Thermo Fisher Scientific*, USA), Capricorn (*Capricorn Scientific*, Germany).

Supplements: Cell Boost 6 Supplement (*Cytiva*, USA), Cell Boost 5 Supplement (*Cytiva*, USA), Cell Boost 7 A (*Cytiva*, USA), Cell Boost 7 B (*Cytiva*, USA), Cosmos Flora Bio Feed A (*Flora Bio*, Turkey), Cosmos Flora Bio Feed B (*Flora Bio*, Turkey), Capricorn's CHO Feed 1 (*Capricorn Scientific*, Germany), Capricorn's CHO Feed 2 (*Capricorn Scientific*, Germany). Protein A (ProA) Biosensors (*Forte Biosciences*, USA).

Culture vessels: 250 mL Erlenmeyer flasks (*Corning*, USA), disposable mini bioreactors for Ambr® 250 Disposable bioreactors system (*Sartorius*,

Germany), disposable culture bags: Flexsafe® RM 20 opt (*Sartorius*, Germany), Flexsafe® STR 200 (*Sartorius*, Germany).

Equipment

TC20 Cell Counter (*Bio-Rad*, USA), Celltron shaker (*Infors HT*, Switzerland), Biostat® RM 20 basic wave-mixed bioreactor (*Sartorius*, Germany), Ambr® 250 mini bioreactor (*Sartorius*, Germany), STR 200 stirred-tank bioreactor (*Sartorius*, Germany), Cedex® Bio Analyzer (*Roche*, Switzerland), Seven Compact S210 pH meter (*METTLER TOLEDO*, USA), Octet® RED96e molecular interaction analyzer (*Forte Biosciences*, USA).

Methods

Cell density measurement

A 20 µL suspension culture of CHO cells was taken. The obtained cell suspension was mixed with trypan blue solution (an acidic aniline dye used for counting the number of dead cells in suspension, which, unlike live cells, are stained blue) in the ratio of 1 : 1. The total volume of the stained suspension should be at least 30 µL to fill two chambers of the slide. The stained suspension was transferred by pipetting. We took 10 µL of the stained suspension with an automatic pipette and, holding the pipette at a 45° angle, gently introduced the suspension into each of the slide chambers. Slides completely filled with stained cells were transferred to an automated TC20 Cell Counter to measure cell density.

Measurement of antibody concentration

The amount of antibodies in the samples was determined by biolayer interferometry (BLI) using Octet® RED96e system (*Forte Biosciences*, USA) and biosensors with immobilized protein A (*Forte Biosciences*, USA), according to the manufacturer's protocol. GamP2C5 antibody solutions obtained earlier by stable transfection and purified by affinity chromatography on Mabselect SuRe™ sorbent (*Cytiva*, USA) as previously described [13, 15, 17] were used as standard samples with known concentrations.

Measurement of residual metabolites

Concentrations of major cultures (glucose, glutamine, and glutamate) and metabolites (lactate and ammonium) in the samples were determined using a Cedex® Bio Analyzer (*Roche*, Switzerland) according to the manufacturer's protocol.

Cell cultivation

The cells were cultured in Erlenmeyer flasks and in wave-mixed bioreactor and stirred-tank bioreactor. Culture media in combination with supplements were used to select optimal conditions (Table 1).

Table 1. Plan of experiment

Experiment number	Media	Supplement	Containers and equipment	Volume, L
1	SFM4CHO	Cell Boost 6	Erlenmeyer flasks	0.25
2	ActiPro™	Cell Boost 7A	Erlenmeyer flasks	0.25
			Biostat® RM 20 bioreactor	10
			Ambr® 250 mini bioreactor	0.20
			Bioreactor STR 200	200
3	BalanCD®	Cell Boost 7A	Erlenmeyer flasks	0.25
		Cell Boost 7B	Biostat® RM 20 bioreactor	10
4	Cosmos	Feed A	Erlenmeyer flasks	0.25
		Feed B	Biostat® RM 20 bioreactor	10
5	Capricorn	Feed 1	Erlenmeyer flasks	0.25
		Feed 2	Biostat® RM 20 bioreactor	
6	Dynamis™ AGT™	Cell Boost 7A	Erlenmeyer flasks	0.25
		Cell Boost 7B		

Cultivation of cells in Erlenmeyer flasks

Cell suspension culture of CHO GamP2C5 (clone 78) cells was performed in Erlenmeyer flasks with a ventilated lid of 250 mL. Initial volume of cell suspension was 30 mL. Initial concentration was 0.3 mL/mL. Starting from the third day of culturing, supplements were added in the amount recommended by the manufacturer. Cultivation was performed at 37°C, 80% humidity and 5% CO_2, with a platform stirring speed of 110–130 rpm at an amplitude of 25 mm. Glucose was maintained at 4.5–5.0 g/L. If necessary, pH was maintained at 6.9–7.4 using 7.5% sodium bicarbonate solution (*Labochem International*, Germany).

Cell cultivation in a Biostat® RM 20 wave bioreactor

The suspension was cultured in wave-mixed bioreactor with a working volume of up to 10 L (Table 2).

The initial working volumes were 25–30% of the final volume, with a minimum concentration of 0.3 mln/mL. On the third day of cultivation, the inoculum was diluted with fresh culture medium to 65–70% of the final volume. Starting on the fifth day of culturing, supplement was added at a concentration according to the manufacturer's instructions. Furthermore, pH 6.8–7.5 was adjusted by adding sodium bicarbonate (*Labochem International*, Germany) as needed. Glucose concentration was maintained at 4.5–5.0 g/L, so glucose solution (*Merck*, USA) was used if necessary. In order to obtain the maximum content of the target product, a change in the temperature parameter and pH at the time of stationary phase of cell cultivation was applied (Table 3).

Cell cultivation in Ambr® 250 mini bioreactors

The suspension was cultured in mini stirred-tank bioreactors with a working volume of up to 250 mL. The initial working volumes were 25–30% of the final volume,

Table 2. Parameters of the cultivation process in the Biostat® RM 20 wave-mixed bioreactor

Parameter	Value
Temperature	37.0 ± 1.0°C
Dissolved oxygen, %	No lower than 40
pH	6.8–7.5
CO_2 concentration in the gas phase, %	5.0 ± 2.0
Platform tilt angle, °	6–10
Platform swing frequency, swing/min	14–30
Volumetric flow rate of gas mixture, L/min	0.2–0.8

Table 3. Parameters of the cultivation process depending on the experiment in the Biostat® RM 20 wave-mixed bioreactor

Parameter	Experiment 1	Experiment 2	Experiment 3
Temperature, °C	37.0	33.0	37.0
Dissolved oxygen, %	40	40	40
pH	7.2	7.2	6.8
CO_2 concentration in the gas phase, %	5.0	5.0	5.0
Platform tilt angle, °	7	7	7
Platform swing frequency, swing/min	15	15	15
Volumetric flow rate of gas mixture, L/min	0.5	0.5	0.5

Table 4. Parameters of the cultivation process depending on the experiment in the Ambr® 250 mini bioreactor

Parameter	Experiment 1	Experiment 2	Experiment 3
Temperature, °C	37.0	33.0	37.0
Dissolved oxygen, %	40	40	40
pH	7.2	7.2	6.8
CO_2 concentration in the gas phase, %	7.0	7.0	7.0
Stirrer, rpm	300–500	300–500	300–500
Air overlay, mLpm	4	4	4
Air sparger, mL/min	4	4	4
O_2 sparger, mLpm	0–2	0–2	0–2

and the minimum concentration was 0.6 mln/mL. On the third day of cultivation, the inoculum was diluted with fresh culture medium to 65–70% of the final volume. Starting on the fifth day of culturing, supplement was added at a concentration according to the manufacturer's instructions. The pH 6.8–7.5 was maintained by adding sodium bicarbonate (*Labochem International*, Germany) as needed. Glucose concentration was maintained at 4.5–5.0 g/L, so glucose solution (*Merck*, USA) was used if necessary. In order to obtain the maximum content of the target protein, the temperature parameter and pH were varied during cultivation (Table 4).

Cell cultivation in the STR 200 stirred-tank bioreactor

The suspension was cultured in a stirred-tank bioreactor with a working volume of 200 L. The initial working volumes were 25–30% of the final volume, and the minimum concentration was 0.5 mln/mL. On the third day of cultivation, the inoculum was diluted with fresh culture medium to 65–70% of the final volume. Starting on the fifth day of culturing, supplement was added at a concentration according to the manufacturer's instructions. The pH 6.8–7.5 was maintained by adding sodium bicarbonate (*Labochem International*, Germany) as needed. Glucose concentration was maintained at 4.5–5.0 g/L, so glucose solution (*Merck*, USA) was used if necessary. In order to obtain the maximum content of the target antibody, the temperature parameter was changed during cultivation (Table 5).

RESULTS AND DISCUSSION

A panel of single-domain antibodies specific to the receptor-binding domain (RBD) of the S-protein of SARS-CoV-2 virus was obtained at the immunobiotechnology laboratory of the Gamaleya National Research Center for Epidemiology and

Table 5. Parameters of the cultivation process in an STR 200 stirred-tank bioreactor

Parameter	Experiment 1
Temperature, °C	37.0–33.0
Dissolved oxygen, %	40
pH	7.2
CO_2 concentration in the gas phase, %	5.0
Stirrer, rpm	60–110
Air overlay, Lpm	10
Air sparger, L/min	10
O_2 sparger, Lpm	0–10

Microbiology by immunization of the two-humped camel with recombinant RBD of the S-protein of SARS-CoV-2 virus and phage display technology [19]. Several of the most promising clones were selected from the obtained panel and subsequently modified by fusion with the Fc fragment of human IgG1. These forms were further investigated in detail by various *in vitro* methods, including direct viral neutralization on live SARS-CoV-2 virus. As a result, a single-domain antibody fused to the Fc fragment of human IgG1 GamP2C5 was obtained (Fig. 1).

A genetic construct was then developed, in order to allow expression of this single-domain antibody fused to the Fc fragment of human IgG1. Today, most therapeutic antibodies are produced in mammalian cells. They are best suited for the production of recombinant antibodies with the highest degree of biological similarity to antibodies produced in the human body [20]. In this study, CHO cells were used. Further, a clone stably producing this antibody was selected using cell-to-cell transfection methods and several rounds of selection. As a result, the cell line CHO GamP2C5 (clone 78) was obtained, expressing a single-domain antibody fused to the Fc fragment of human IgG1 GamP2C5.

The most important component for the development of culturing technology is the culture medium which ensures cell viability and proliferation. The composition of the culture medium directly affects the production rate and quality of biopharmaceuticals. Therefore, it is important for researchers working with cell

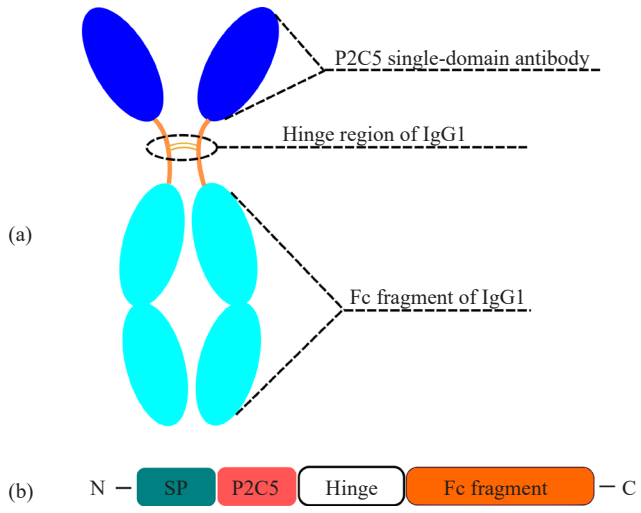

Fig. 1. (a) Schematic representation of a single-domain antibody fused with the Fc fragment of human IgG1 GamP2C5; (b) structure of a single-domain antibody fused with the Fc fragment of human IgG1 (SP—amino acid sequence of the signal peptide of the heavy chain of IgG1; P2C5—amino acid sequence of the P2C5 single-domain antibody; Hinge—amino acid sequence of the hinge region of IgG1; Fc fragment—amino acid sequence of the Fc fragment of IgG1)

cultures to select the right medium which is suitable for their purposes [21]. In order to select the optimal culture media based on availability and affordability, 6 basic culture media and 7 culture supplements were analyzed (Fig. 2).

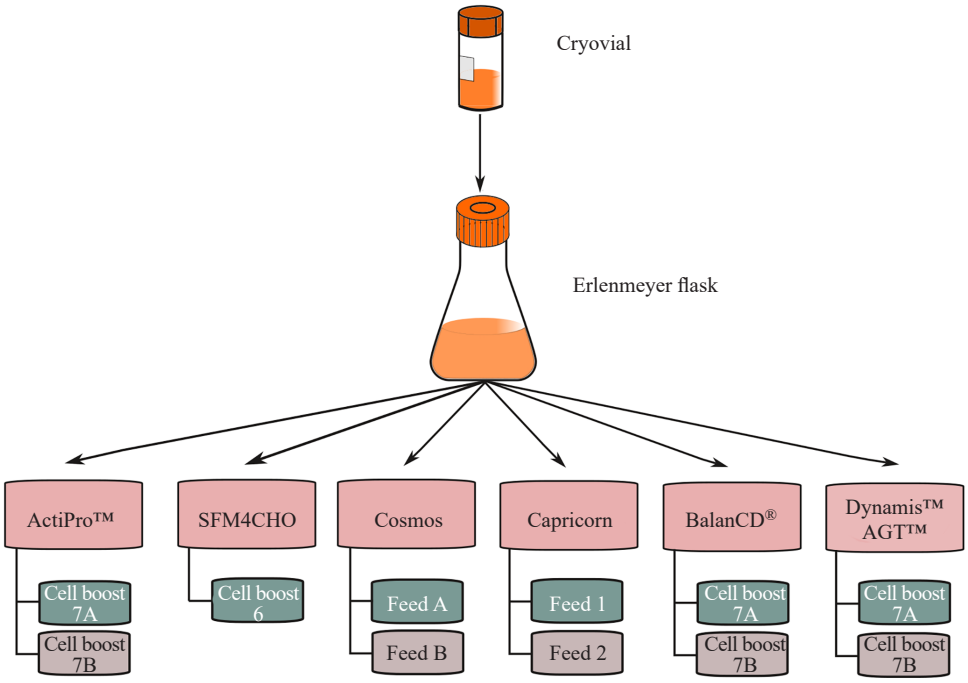

Fig. 2. Combination of culture media and supplement during the development of the cultivation process

Using these culture media and supplements, an experiment was conducted in three repetitions, aimed at developing a process for culturing the resulting clone in flasks to obtain the highest content of the target product. Cells were cultured with uniform initial parameters. The experiment was started at a cell density of 0.3 mln/mL. Cells were cultured in fed-batch mode, with glucose and glutamine levels controlled. The survival ratios and cell culture densities at a particular day of culturing are shown in Fig. 3.

The leader in cell density was ActiPro™ medium. Cells on this medium reached a density of 10 mln/mL. Cosmos and BalanCD® media showed the best results in terms of the longest duration of cultivation. On both

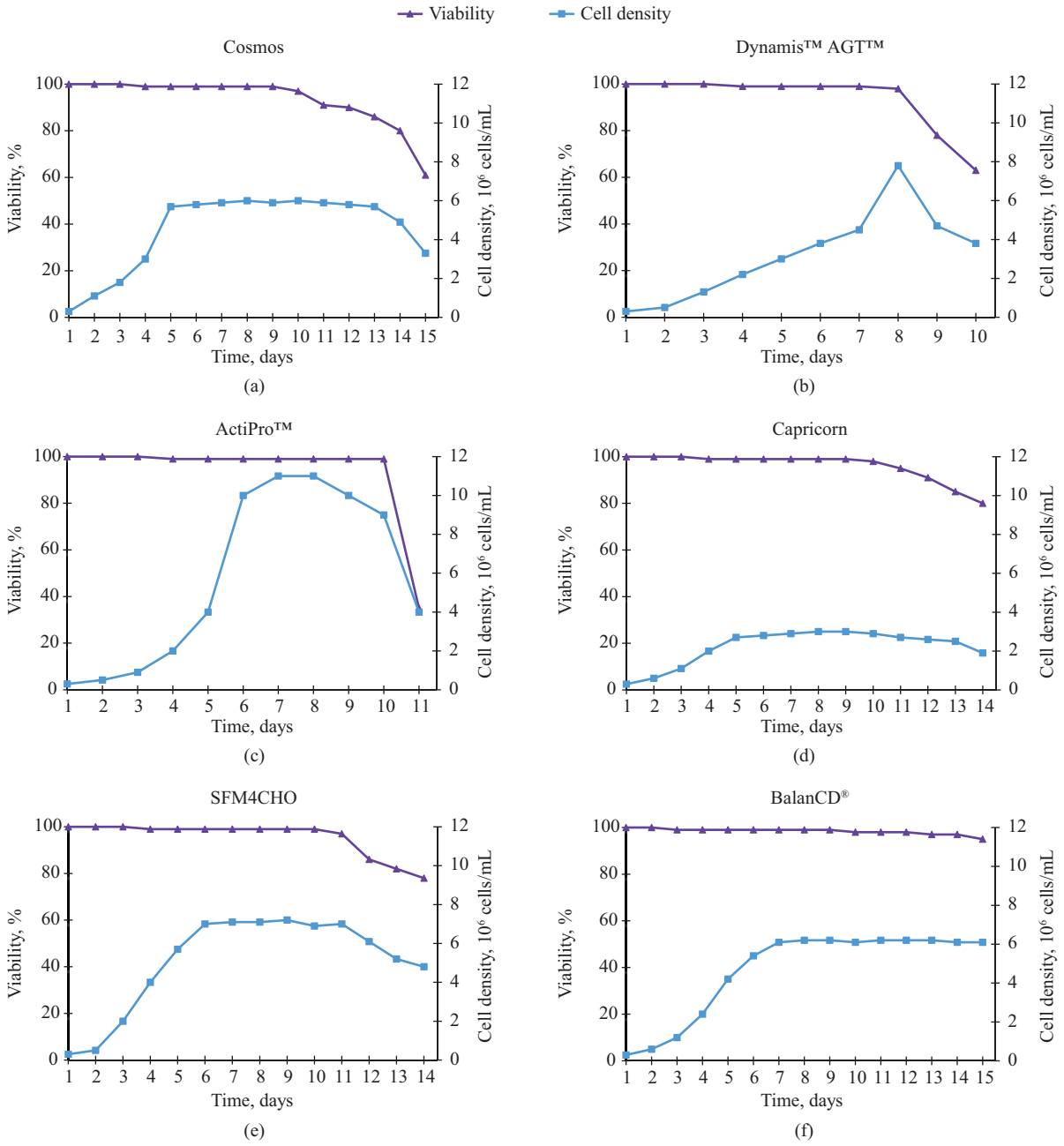

Fig. 3. The ratio of survival rate and cell culture density of CHO GamP2C5 (clone 78) in an Erlenmeyer flask when cultivated on various culture media:
(a) Cosmos;
(b) Dynamis™ AGT™;
(c) ActiPro™;
(d) Capricorn;
(e) SFM4CHO;
(f) BalanCD®

Table 6. Yield of the target antibody GamP2C5 determined using an Octet® RED96e system

Culture media	SFM4CHO	ActiPro™	Cosmos	BalanCD®	Dynamis™ AGT™	Capricorn
Product yield, μg/mL	364.8	671.0	478.0	580.0	284.0	220.0

media, the cell culture showed a high level of viability for 15 days. The highest content of target antibody was recorded on ActiPro™ culture medium. The content amounted to 671 μg/mL (Table 6).

Based on the growth graph of cell suspension on different culture media and based on the obtained values of final target protein content, three best culture media—ActiPro™, BalanCD®, Cosmos—were selected to develop further culturing process in wave bioreactors.

In order to study the cultivation process of the obtained clone, it was necessary to develop the cultivation process in wave bioreactors with selection of different conditions. For the purposes of this study, experiments were conducted with three best culture media. The experiments were conducted under identical conditions in a wave type stirred bioreactor with a working volume of 10 L (Fig. 4).

The initial cell density was 0.3 mln/mL. The ratio of survival and cell culture density on a particular day of cultivation is shown in Fig. 5.

In this experiment, ActiPro™ culture medium was the leader in terms of cell density, culturing time, and target protein content. Cells on this culture medium reached a density of 10 mln/mL. They were cultured for 10 days, and the target protein content was 440 μg/mL. Comparison of GamP2C5 target antibody content depending on the culture medium is described in Table 7.

After analyzing three basic culture media, ActiPro™ medium was selected to show the maximum result in terms of target product productivity. Next, it was necessary to select the optimal cultivation conditions for further scaling of the process (Fig. 6).

In this experiment, different cultivation parameters were worked out in a wave bioreactor with a working volume of 10 L. In this part of the work, the productivity of the target protein was compared after changing the temperature parameter and pH during cultivation. The survival ratio and cell culture density on a particular day of cultivation are shown in Fig. 7.

When the temperature was lowered to 33°C, the culturing time increased, but the target protein content was lower than that at 37°C. Culturing the cells at pH 6.8 showed a lower result in target protein content compared to culturing at pH 7.2. When used on Biostat® RM 20 wave type stirred bioreactors, temperature and pH shifts did not show the expected result. The best productivity result was obtained after

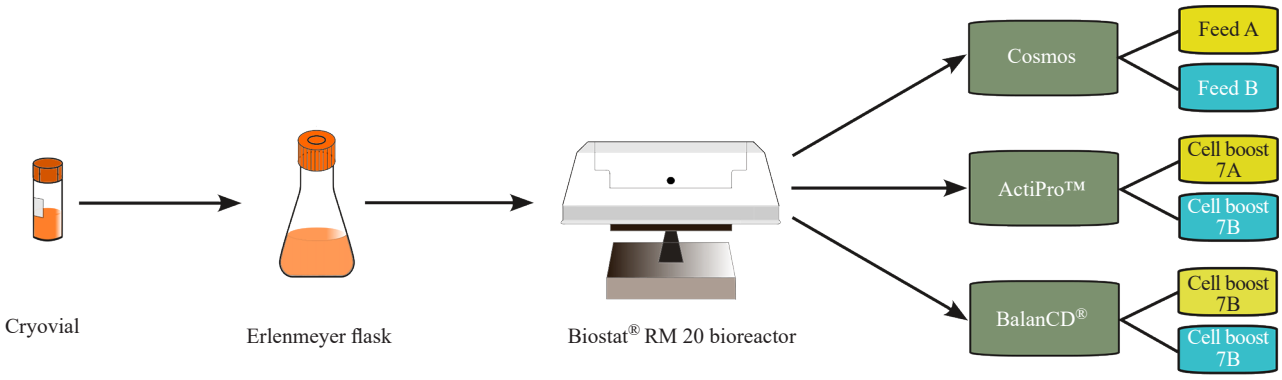

Fig. 4. Combinations of culture medium and supplement for the cultivation process in Biostat® RM 20 wave bioreactors

Table 7. Yield of the target antibody GamP2C5 determined using an Octet® RED96e system

Culture media	ActiPro™	Cosmos	BalanCD®
Product yield, μg/mL	440	250	325

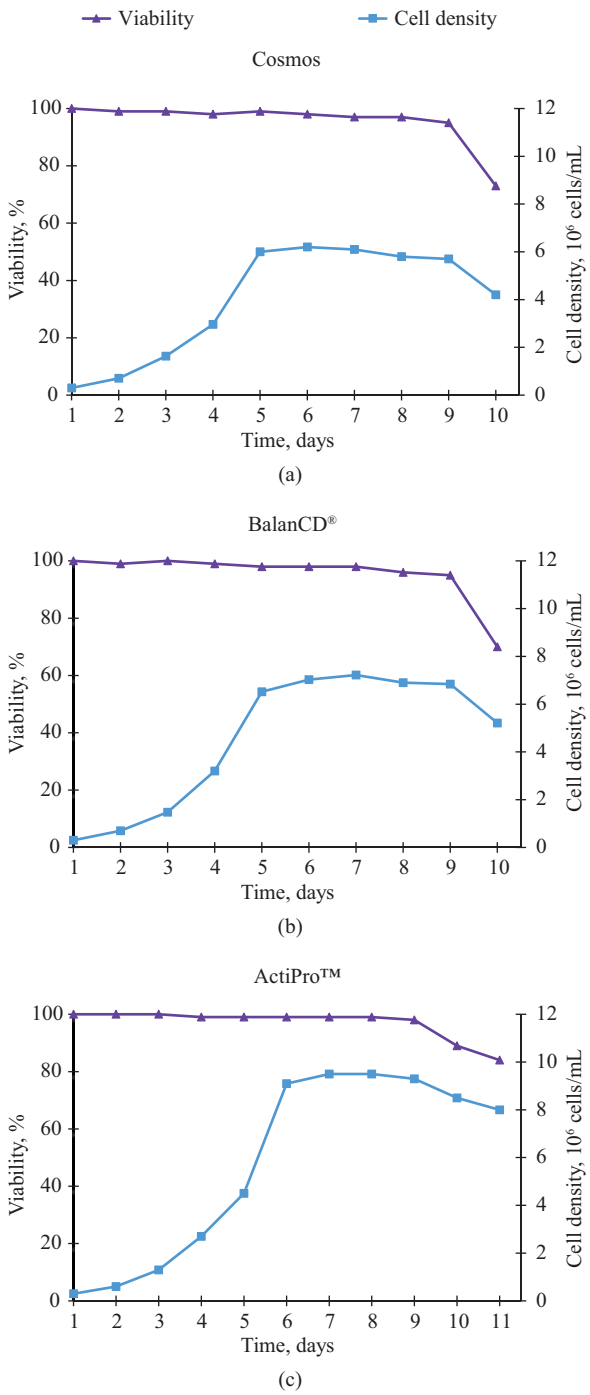

Fig. 5. The ratio of survival rate and cell culture density of CHO GamP2C5 (clone 78) in the Biostat® RM 20 wave bioreactor when cultivated on various culture media: (a) Cosmos; (b) BalanCD®; (c) ActiPro™

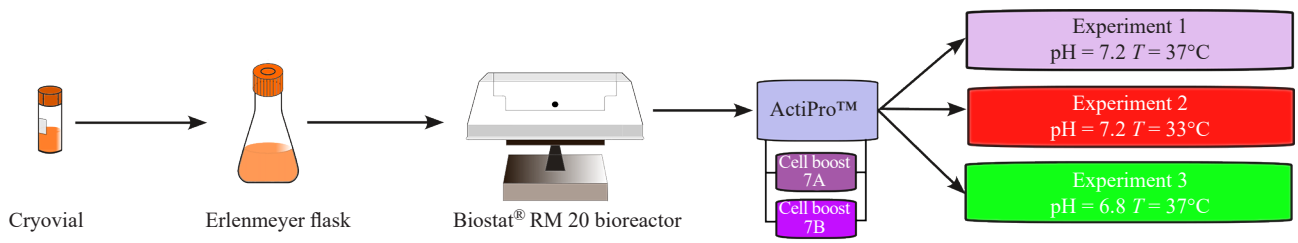

Fig. 6. Selection of optimal parameters for the cultivation process in RM 20 wave bioreactors

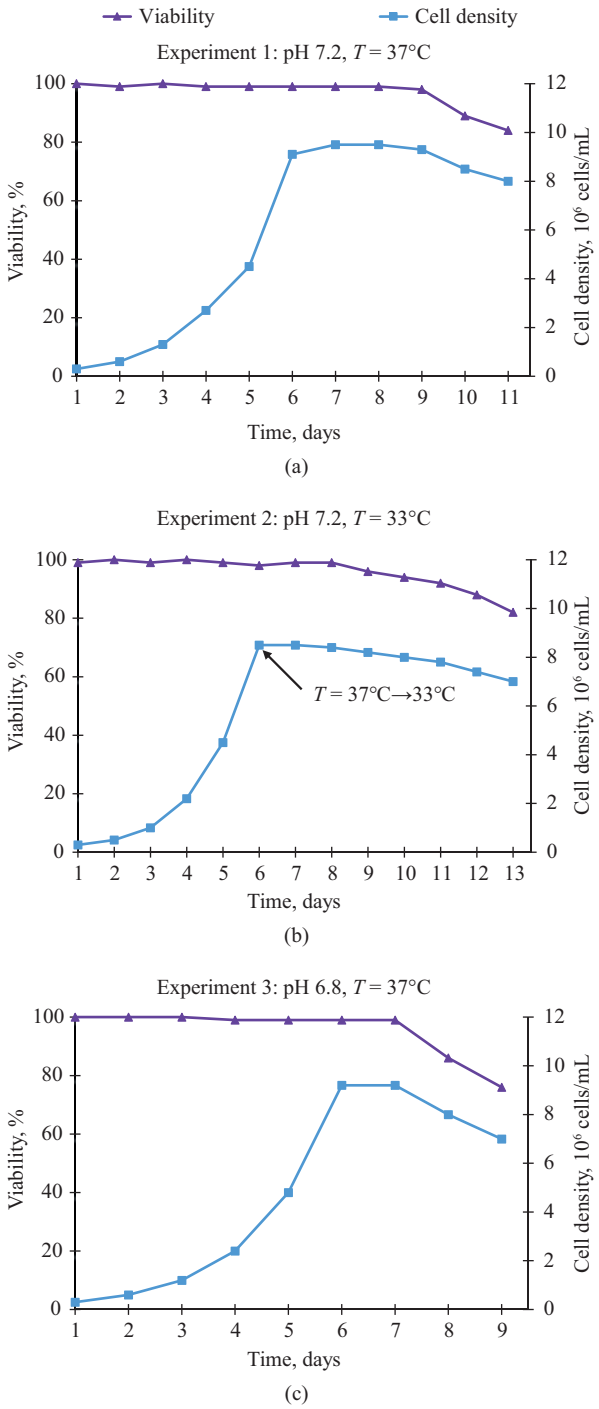

Fig. 7. Cultivation of CHO GamP2C5 (clone 78) cells under various cultivation conditions in Biostat® RM 20 wave bioreactor: (a) experiment 1: pH 7.2, $T = 37$°C; (b) experiment 2: pH 7.2, $T = 33$°C; (c) experiment 3: pH 6.8, $T = 37$°C

culturing at 37°C and pH 7.2. The amount of the target antibody GamP2C5 is summarized in Table 8.

Thus, as a result of the work done at this stage, we have developed a process for culturing this clone in a laboratory-scale wave bioreactor (working volume of 10 L) with the cultivation regime indicated in Table 9.

After analyzing the process on ActiPro™ medium with three different cultivation conditions, we selected the parameters that showed the maximum result in terms of target antibody production. Then we scaled up to a STR 200 stirred-tank bioreactor. For this process, the optimal conditions for cell cultivation had to be selected, while taking into account the change of

Table 8. Yield of the target antibody GamP2C5 determined using an Octet® RED96e system

Number of experiment	Experiment 1	Experiment 2	Experiment 3
Product yield, μg/mL	440	374	223

Table 9. Cultivation parameters for CHO cells on ActiPro™ medium in the Biostat® RM 20 wave bioreactor

Parameter	Value
Temperature, °C	37.0
Dissolved oxygen, %	40
pH	7.2
CO_2 concentration in the gas phase, %	5.0
Platform tilt angle, °	7
Platform swing frequency, swing/min	15
Volumetric flow rate of gas mixture, L/min	0.5
Start adding supplements, day	5

stirring type. The Ambr® 250 mini bioreactor system was used for the experiments (Fig. 8).

For the processes in mini bioreactors, we used ActiPro™ culture medium in combination with supplements 7A and 7B. After the experiment in flasks, they showed the best result in terms of target antibody yield (671 μg/mL), and the highest cell density (10 mln/mL) was recorded on this medium. Furthermore, after testing the conditions on the Biostat® RM 20 bioreactor with wave-type stirring, the ActiPro™ culture medium also showed the best result in terms of target antibody content, which amounted to 440 μg/mL.

In experiments in Ambr® 250 mini bioreactors, the productivity of the target protein was compared after changing the temperature parameter and pH during cultivation. The survival ratio and cell culture density on a certain day of cultivation are presented in Fig. 9.

In these experiments, it was found that when the temperature was lowered to 33°C, the culturing time and target protein content were longer than at 37°C. Culturing the cells at pH 6.8 showed a lower yield of target protein when compared to culturing at pH 7.2. The experiment with pH variation did not show the expected results as with the experiment in the Biostat® RM 20 wave type stirred bioreactor. However, the temperature variation in this experiment showed the best result in terms of target protein content. The amount of target antibody GamP2C5 is presented in Table 10.

Taking into account the results obtained from the experiments on Ambr® 250 mini bioreactors, process

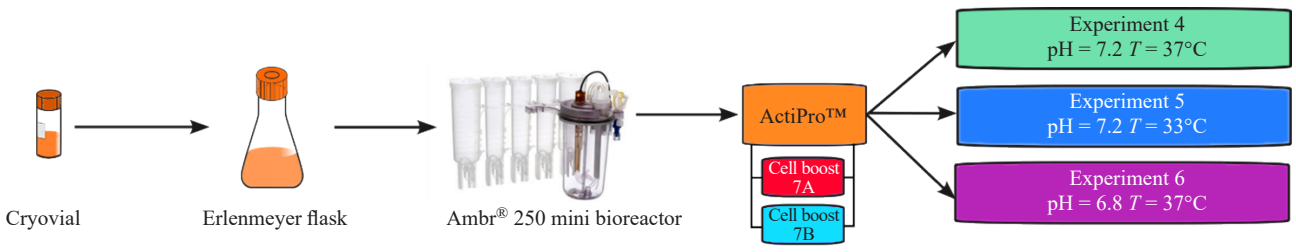

Fig. 8. Selection of optimal parameters for the cultivation process in Ambr® 250 mini bioreactors

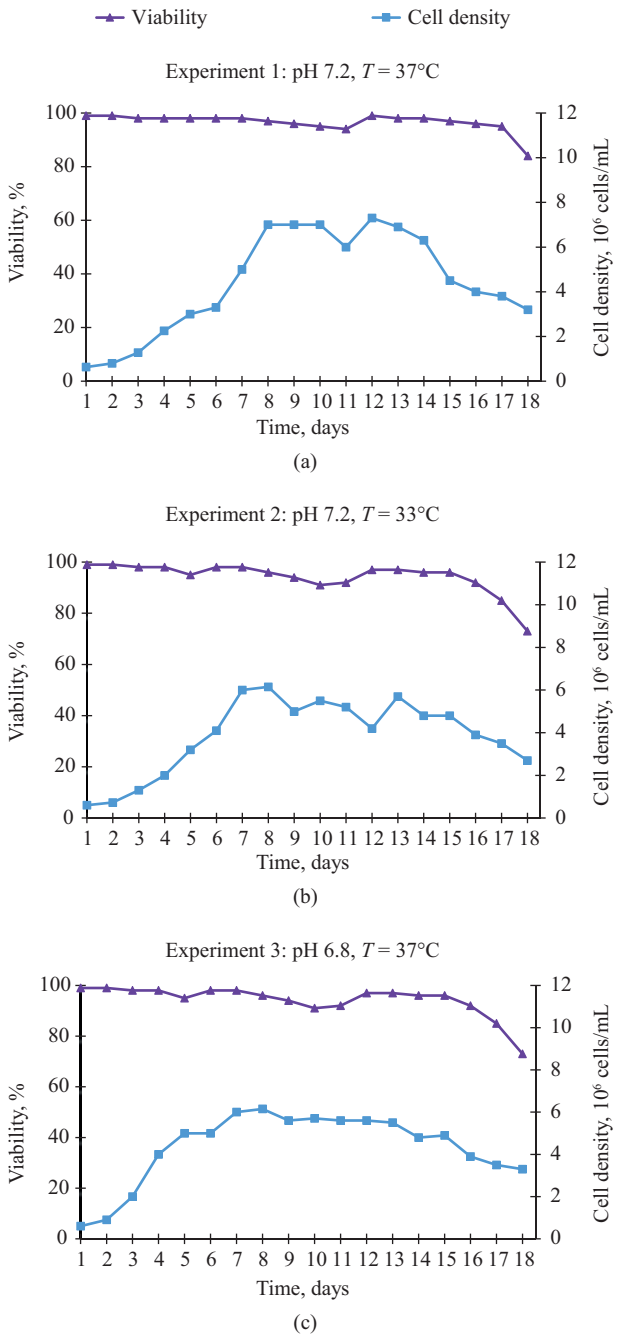

Fig. 9. Cultivation of CHO GamP2C5 (clone 78) cells under various cultivation conditions in Ambr® 250 mini bioreactors:
(a) experiment 1: pH 7.2, $T = 37$°C;
(b) experiment 2: pH 7.2, $T = 33$°C;
(c) experiment 3: pH 6.8, $T = 37$°C

Table 10. Yield of the target antibody GamP2C5 determined using an Octet® RED96e system

Number of experiment	Experiment 4	Experiment 5	Experiment 6
Product yield, μg/mL	382	456	240

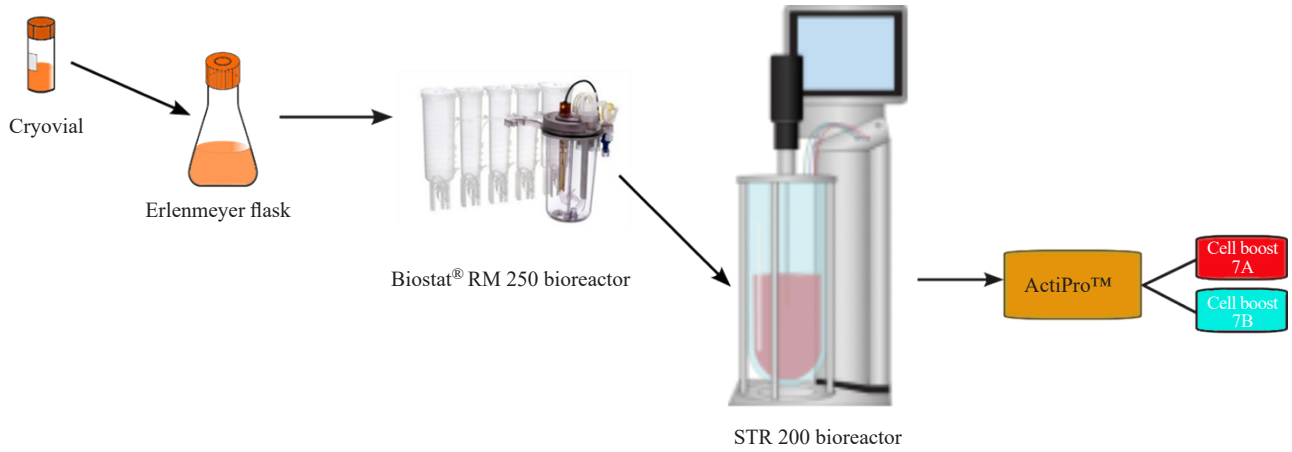

Fig. 10. Scaling diagram for the cultivation process to an STR 200 stirred-tank bioreactor with a working volume of 200 L

parameters were selected for the STR 200 stirred-tank bioreactor (Fig. 10).

After conducting a number of experiments using Ambr® 250 mini bioreactors on ActiPro™ culture medium in combination with supplements 7A and 7B, the conditions for optimal pH and temperature were selected. They showed the maximum result in terms of target antibody productivity. For the STR 200 stirred-tank bioreactor with a working volume of 200 L, the parameters obtained from the processes in the Ambr® 250 mini bioreactor were used. In order to scale the process parameters from Ambr® 250 mini bioreactors to STR 200 bioreactor, modeling was performed using Sartorius software[1]. This performs the transfer of culturing process parameters from a small volume bioreactor to a larger one while maintaining mass transfer characteristics. In normal conditions, one of the dimensionless scaling factors used to scale up/down bioreactor processes is the volume flow rate *(vvm*, where *v* is the volume of air in liters, *v* is the volume of medium in liters, *m* is the time in minutes during which the exchange process (aeration) takes place) [22]. Taking this parameter into account, scaling was performed (data not presented). Furthermore, measurements on residual metabolites (glucose, glutamine, glutamate, lactate, and ammonium) were performed to ensure prolonged proliferation and block cell apoptosis during supplementation from the fifth day of culturing (Table 11).

The survival ratio and cell culture density on a particular day of cultivation are shown in Fig. 11.

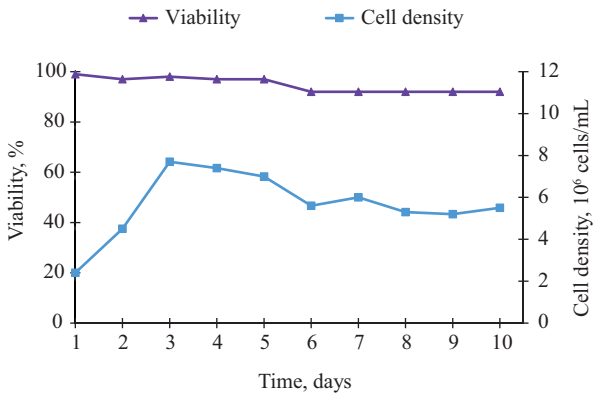

Fig. 11. Cultivation of CHO GamP2C5 (clone 78) cells in ActiPro™ medium in an STR 200 stirred-tank bioreactor

After the process was carried out in the STR 200 stirred-tank bioreactor with cultivation modes selected using the Ambr® 250 mini bioreactor, the amount of the target antibody product GamP2C5 amounted to 564.4 μg/mL. This value exceeds the initial value (456 μg/mL) obtained by culturing in the Ambr® 250 mini bioreactor.

CONCLUSIONS

Work was carried out to enable the selection of optimal parameters of the cultivation process for the production of component I of the GamCoviMab candidate drug. Component I of the GamCoviMab candidate drug is an antibody GamP2C5 (CHO cell culture, clone 78). For this clone, the optimal culturing

[1] https://www.sartogosm.ru/. Accessed June 01, 2022.

Table 11. Data of residual metabolites according to the Cedex® Bio Analyzer device

Days	Glucose, mg/L	Glutamine, mM/L	Glutamate, mg/L	Lactate, mg/L	Ammonium, mM/L	Addition
5	2000.74	0.32	381.32	2345.45	7.920	1.0% 7A 0.1% 7B 1 g/L glucose
6	3343.45	0.15	598.39	1806.77	10.148	1.50% 7A 0.15% 7B 200 mM glutamine 2 g/L glucose
7	1721.22	0.48	972.53	2065.86	11.544	2.0% 7A 0.2% 7B 200 mM glutamine 3 g/L glucose
8	6158.21	1.23	1320.79	300.88	8.311	2.50% 7A 0.25% 7B
9	3891.29	0.59	1402.18	650.86	10.032	3.0% 7A 0.3% 7B
10	2191.10	0.52	1410.47	873.21	9.716	1.50% 7A 0.15% 7B
11	2272.02	0.11	584.63	727.05	3.759	2.0% 7A 0.2% 7B
12	323.82	0.21	609.07	257.12	3.258	2.50% 7A 0.25% 7B 4 g/L glucose
13	3783.16	0.21	1021.88	242.33	3.358	2 g/L glucose
14	4548.37	0.89	1101.30	323.79	8.285	–

process parameters were achieved in a stirred-tank bioreactor at pH 7.2 using temperature reduction from 37 to 33°C, with ActiPro™ culture medium in combination with culture additives 7A and 7B, in which the CHO cell culture expressed the highest amount of the target antibody. This conclusion was based on a study of the culture conditions of CHO cells stably producing the GamP2C5 antibody (clone 78). The process of cultivation of this clone in a wave bioreactor with a working volume of 10 L is described. The optimal culture medium was selected, in order to give the maximum content of the target protein. Experiments were carried out to evaluate the effect of temperature variation during cultivation on the productivity of the target antibody. Furthermore, the pH of the culture medium was selected; something which also affects the cultivation of this clone. Then, for transfer to a production scale bioreactor (stirred-tank bioreactor), the optimal parameters were selected taking into account the change of stirring type. The Ambr® 250 mini bioreactor system was used for this work, by means of which the optimal parameters in terms of temperature and pH were selected. Furthermore, the study revealed that the productivity indices for the CHO cell line stably producing the GamP2C5 antibody (clone 78) have different values in a wave-mixed and stirred-tank bioreactors. For this clone in a bioreactor with wave-type agitation (working volume 10 L), the best result in the yield of target antibody was demonstrated at T = 37°C. When cultured in a stirred-tank bioreactor (Ambr® 250 mini bioreactor) the highest productivity was demonstrated at T = 33°C. At the same time the content of target antibody in Ambr® 250 mini bioreactor at T = 33°C exceeds this index when compared with the bioreactor with wave type of agitation (working volume 10 L) at T = 37°C. Cultivation was carried out in a stirred-tank bioreactor with a working volume of 200 L, after selecting the optimal cultivation parameters for the CHO cell line

stably producing the GamP2C5 antibody (clone 78). The parameters for this process were selected using data obtained from experiments in an Ambr® 250 mini bioreactor. Furthermore, supplements were added with metabolite assay/control, in order to ensure optimal culture conditions for CHO cells stably producing GamP2C5 antibody. Thus, in our study, we developed an efficient technology for culturing CHO cells stably producing GamP2C5 antibody as component I of the GamCoviMab candidate drug for emergency prevention and therapy of infection caused by SARS-CoV-2 virus.

Authors' contributions

D.S. Polyansky—conducting the experiments of cultivating CHO cells in an Ambr® 250 mini bioreactor, conducting the experiments of scaling the cultivation process, data collection and analysis, and writing the text of the manuscript.

E.I. Ryabova—development of CHO cell line, stably producing GamP2C5 modified single-domain antibody, conducting the experiments of the selection of culture media in Erlenmeyer's flasks, and data collection and analysis.

A.A. Derkaev—conducting the experiments of the selection of cultural media in Erlenmeyer's flasks, measuring the concentration of the product in the culture media, and data collection and analysis.

N.S. Starkov—scaling the cultivation process in an STR 200 stirred-tank bioreactor and preparation of materials for the manuscript.

I.S. Kashapova—conducting the experiments on cultivation in Biostat® RM 20 wave bioreactors.

D.V. Shcheblyakov—design of a genetic construct expressing a modified single-domain antibody.

A.P. Karpov—managing the experiments on scaling the cultivation process; correcting the manuscript text, and approval of the final version of the manuscript for publication.

I.B. Esmagambetov—general management, correcting the manuscript text, and approval of the final version of the manuscript for publication.

The authors declare no conflicts of interest.

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