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Development of cell lines producing recombinant therapeutic proteins, with denosumab as a case study

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ABSTRACT

INTRODUCTION. A key factor in the creation of biotechnological medicinal products is to establish cell lines for high-yield production of recombinant proteins. The development of selection protocols and highly efficient screening approaches for cell lines producing target proteins is a necessary step in the development of recombinant technology for high-yield target protein production.

AIM. This study aimed to derive producer cell lines from a CHO suspension cell line for high-yield production of the recombinant monoclonal antibody denosumab.

MATERIALS AND METHODS. A CHO-K1 suspension cell line was cultured using serum- and animal component-free media and feeds. The cells were transfected with plasmids containing light and heavy chains of denosumab by electroporation using a MaxCyte STX system. The transfected cells were selected under antibiotic pressure (hygromycin and geneticin). Monoclonal cell lines were obtained using a ClonePix FL system. Leader monoclonal cell lines were identified by determining denosumab concentrations by enzyme-linked immunosorbent assay (ELISA) following fed-batch culture.

RESULTS. The optimum concentrations of antibiotics for the selection of CHO-derived denosumab-producing cell lines were 600 mg/L for hygromycin and 600 mg/L for geneticin. The selection process following transfection was successful in 1,041 (about 54%) of 1,920 minipools. Denosumab-producing minipools were identified by screening culture fluid samples from 96-, 24-, and 6-well plates using ELISA. Then, 23 leader minipools were chosen and adapted to suspension culture in shaker flasks. The growth and production characteristics of these 23 minipools indicated the leader minipool for cloning (mp-19). This minipool provided a denosumab yield of 1.92 g/L on day 7 of fed-batch culture. Using mp-19, the authors obtained monoclonal cell lines providing up to 6.5 g/L denosumab yields on day 9 of fed-batch culture.

CONCLUSIONS. The authors obtained monoclonal cell lines for high-yield denosumab production. The offered approach to producer cell line development can be applied to the production of various recombinant proteins, including monoclonal antibodies, enzymes, and blood coagulation factors.

Keywords: Chinese hamster ovary cells; CHO; producer cell lines; recombinant protein; denosumab; ELISA; therapeutic monoclonal antibodies; biotechnological medicinal product

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Разработка клеточных линий-продуцентов рекомбинантных терапевтических белков на примере деносумаба

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РЕЗЮМЕ

ВВЕДЕНИЕ. При создании биотехнологических лекарственных препаратов ключевое значение имеет разработка высокопродуктивных клеточных линий-продуцентов рекомбинантных белков. Отработка протоколов селекции и подходов для эффективного скрининга линий-продуцентов целевых белков – необходимый этап разработки технологии производства рекомбинантных белков с высоким выходом целевого продукта.
ЦЕЛЬ. Получение высокопродуктивных клеточных линий-продуцентов рекомбинантного белка деносумаба на основе суспензионной клеточной линии CHO.
МАТЕРИАЛЫ И МЕТОДЫ. Использовалась суспензионная клеточная линия CHO-K1. Для культивирования применялись среды и подпитки без использования сыворотки или других компонентов животного происхождения. Клетки линии CHO трансфицировали плазмидами, содержащими гены легкой и тяжелой цепей деносумаба, с использованием метода электропорации с помощью системы MaxCyte STX. Селекцию трансфицированных клеток проводили с добавлением антибиотиков (гигромицин, генетицин). Моноклональные клеточные линии получали с использованием системы ClonePix FL. Проводили периодическое культивирование с добавлением подпитки и определение концентрации деносумаба методом иммуноферментного анализа (ИФА) в культуральной жидкости для выявления лидерных моноклональных клеточных линий.
РЕЗУЛЬТАТЫ. Оптимальная концентрация антибиотиков для селекции клеточных линий-продуцентов на основе клеток CHO составила 600 мг/л гигромицина и 600 мг/л генетицина. После проведения трансфекции из 1920 минипулов селекцию прошел 1041 (около 54%). Отбор минипулов-продуцентов деносумаба проводили путем скрининга проб культуральной жидкости с использованием ИФА из 96-, 24- и 6-луночных планшетов. Отобранные 23 лидерных минипула были адаптированы к условиям шейкерного культивирования. С учетом показателей ростовых и продукционных характеристик минипулов определен лидерный минипул (mp-19) с продуктивностью 1,92 г/л (на 7 сут периодического культивирования). На основе mp-19 получены моноклональные клеточные линии-продуценты деносумаба с продуктивностью до 6,5 г/л на 9 сут культивирования с подпиткой.
ВЫВОДЫ. Получены высокопродуктивные моноклональные клеточные линии-продуценты деносумаба. Предложенный подход к созданию клеток-продуцентов может быть применен для получения различных рекомбинантных белков, включая моноклональные антитела, ферменты, факторы свертывания крови.

Ключевые слова: клетки яичника китайского хомячка; CHO; клеточные линии-продуценты; рекомбинантный белок; деносумаб; ИФА; терапевтические моноклональные антитела; биотехнологический лекарственный препарат

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INTRODUCTION

Obtaining high-yield cell lines of recombinant proteins is a key step in the development of biotechnological medicinal products [1]. To achieve high expression of recombinant proteins, various approaches are used.

Stable integration of foreign DNA into a host cell genome requires an optimal expression vector including additional genetic elements, such as selection systems, gene amplification mechanisms providing transcriptional activity, as well as genes that regulate cell proliferation (for example, *bcl-2*, *xiap*, *aven*, *mcl-1*) [2, 3].

The choice of a cell line is an essential part of biopharmaceutical development. The majority of the products are primarily based on eukaryotic cell lines like Chinese hamster ovary (CHO) cells, mouse myeloma cells (NS0 and Sp2/0-Ag14), human embryonic kidney cells (HEK 293), and Syrian hamster kidney (BHK line) cells. Eukaryotic cell lines are preferred for their ability to synthesize proteins that are structurally and biochemically similar to those seen on endogenous human proteins [4].

Cell lines with high viable cell density (VCD) are crucial for large-scale commercial production, since they improve the process throughput. Growth media and feed optimisation for certain producer cells is important for a significant increase in cell line yield [5–7]. The use of chemically defined, animal-origin free growth media and additives with a certain chemical formula allows precise control over target product profile such as glycan composition and biological activity, etc.

Optimising various parameters of growth media for producer cells (duration, temperature profile, gas supply, pH control, etc.) is crucial for significantly increasing product yield [8, 9]. Co-expression of additional proteins/enzymes can increase the yield of a target product [10, 11].

The authors analyse the implementation of an approach aimed at developing high-yield cell lines producing recombinant therapeutic proteins using denosumab (manufacturer – JSC GENERIUM) as a case study.

Denosumab is a human monoclonal IgG2 antibody targeting RANKL cytokine, an osteoclast differentiation and activation factor. RANKL inhibition reduces the formation and survival of osteoclasts, thus decreasing bone resorption intensity [12]. Currently, denosumab-based products have been approved and marketed for postmenopausal osteoporosis [13, 14] and cancers (myeloma, bone metastases, unresectable giant cell tumour of bone, and benign bone tumours) [15, 16]. Preclinical and clinical trials of denosumab biosimilars are currently underway[1]. Denosumab biosimilar development is expected to increase the drug availability and accessibility for patients in the Russian Federation, thus facilitating state policies of pharmaceutical industry.

This study aimed to derive producer cell lines from a CHO suspension cell line for high-yield production of the recombinant monoclonal antibody denosumab.

MATERIALS AND METHODS

Materials

CHO-K1 (H-188) cell line was obtained from the cell culture collection of National Research Centre “Kurchatov Institute”, State Research Institute of Genetics, Russia (hereinafter referred to as CHO line) and adapted to suspension cultivation without using serum or any other components of animal origin. The following reagents were used: BalanCD CHO Growth A Medium (BCD) (Fujifilm, Irvine Scientific, USA); BalanCD CHO Feed 4 (Fujifilm, Irvine Scientific, USA); ClonaCell Flex semi-solid cloning medium (Stemcell, Canada); electroporation buffer (MaxCyte, USA); PBS phosphate-buffer saline (Ecoservice, Russia); Twin 20 (Panreac AppliChem, Spain); denosumab reference drug (Prolia, Amgen Europe B.V., The Netherlands); albumin (Sigma-Aldrich, Japan); goat polyclonal antibodies against human

[1] https://dhpp.hpfb-dgpsa.ca/review-documents/resource/RDS1710769045419
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https://adisinsight.springer.com/drugs/800049295

IgG (Sigma, Japan); goat polyclonal antibodies against human IgG conjugated with horseradish peroxidase (Sigma, Japan); 3,3', 5,5' - Tetramethylbenzidine (Transgene Biotech, China); Dimethyl sulfoxide (Sterile Filtered, Cell Culture Tested, CDH, India).

Methods

Obtaining expression vectors. Synthetic codon-optimised sequences of denosumab light and heavy chains were cloned into an expression vector with a selective marker. The obtained plasmids with the light chain (pGNR-FAB) and heavy chain (pGNR-FC) genes were subsequently used for transfection. Gene expression was controlled by a hybrid CMV enhancer/EF1alpha promoter. A hybrid promoter, combining the CMV enhancer with the EF1alpha promoter, was used to control gene expression. A similar expression vector structure was described previously [11].

Suspension cultivation of CHO cells. CHO viable cell density: 0.5×10^6 cell/mL. The cells were cultivated in BalanCD CHO Growth A media at 37 °C and 5% CO_2, with at least 75% humidity; continuous agitation on a shaker at 100 rpm to keep cells suspended and aerated (Infors HT, Multitron, USA).

Determining growth and production parameters of the cell cultures. Viable cell density (VCD) was counted using a 0.1% trypan blue solution on an automatic Countess II FL cell counter (Life Technologies, USA). Cumulative cell density (CCD) and productivity (mg/L) were calculated by the formulas presented in [17, 18].

Transfection of CHO cells using electroporation. The cells were transfected using MaxCyte specific electroporation system, including an OS-100 cuvette by CHO protocol. The cells were centrifuged, then the precipitate cells (pellet) were resuspended in the MaxCyte electroporation buffer. Next, the cell suspension was mixed with pGNR-FAB and pGNR-FC plasmids in an equimolar ratio. In case of a negative electroporation control, the same procedure was repeated without plasmids.

Generation of minipools. Transfected CHO cells were seeded into flat-bottomed 96-well plates (Greiner Bio-One, Austria) 50 µL each in BCD culture medium with selective antibiotics and cultured at 37 °C, 5% CO_2 and 70% humidity. The culture fluid from the minipools was then analysed using enzyme immunoassay (ELISA) after 12 days. The highest productivity minipools were transferred to 24- and 6-well plates (Greiner Bio-One, Austria), and then to Erlenmeyer flasks (Corning, USA) for further adaptation to growth in suspension with constant stirring.

Cloning in a semi-solid medium. ClonaCell Flex semi-solid medium was seeded with the leader minipools in 6-well plates (Greiner Bio-One, Austria). Leader minipools were incubated under static conditions at 37 °C, 5% CO_2 in a CO_2 incubator (Sanyo, Japan) until colonies appeared. The cell lines were selected using the ClonePix FL robotic system (Molecular Devices, USA).

Batch cultivation. Producer cells were cultured in BCD medium by TubeSpin Bioreactor minibioreactors (TPP Techno Plastic Products AG, Switzerland), with a cell concentration of 0.5×10^6 cells/mL. BalanCD CHO Feed 4 was used. Cultivation conditions: CO_2 – 5%, humidity – at least 75%, temperature – 37 °C, and agitation speed of 100 rpm. Cultural fluid was sampled daily starting on cultivation Day 3–4; VCD and viability were calculated as well.

ELISA. Sorption was performed in MaxiSorb plates (Nunc, Denmark) using goat polyclonal antibodies against human IgG at a concentration of 4 µg/mL (carbonate-bicarbonate buffer, pH 9.4) at 37 °C for 1 h. The wells were then washed with PBS supplemented with Tween 20 (PBS-T) and blocked with bovine serum albumin solution in PBS-T (PBS-Ta, Sigma-Aldrich, Japan). The study samples containing denosumab and the reference sample (denosumab, Prolia) were incubated for 1 h at 37 °C on an ELMI ST-3L thermostatic orbital shaker (Elmi, Latvia) at 300 rpm. After washing the wells with PBS-T, detection goat anto-human IgG horseradish peroxidase-conjugated polyclonal antibodies were added at a concentration of 1 µg/mL in PBS-Ta and incubated for 1 h at 37 °C on by ELMI ST-3L shaker at 300 rpm. The wells were then washed with PBS-T, and a tetramethylbenzidine solution (50 µL) was added. Sulfuric acid was used to stop the reaction. Optical density was measured at a wavelength of 450 nm (OD_{450}) using

a Benchmark Plus spectrophotometer (Bio-Rad, Germany).

Cell culture cryopreservation. Antibiotic-free culture medium containing 10% dimethyl sulfoxide was used for cryopreservation of cell cultures (in the logarithmic growth phase). The cell concentration was 5–10 million cells/mL. After slow cooling of the cell-containing ampoules (around (1 °C/min) in a freezer at –80°C in freezing containers (Mr. Frosty, Thermo Scientific), the ampoules were placed in liquid nitrogen vapor.

RESULTS

Figure 1 outlines the process of developing producer cell line for a recombinant protein, using denosumab produced in CHO cells as a case study. This process typically involves the following steps:

- transfecting CHO cells;
- obtaining producer minipools (selecting producers, screening to identify the most productive lines, selecting a leader minipool);
- obtaining producer clones (cloning process, screening the resulting producer cell lines, selecting leader cell lines);
- studying cell line stability and recombinant protein expression in leader producer lines;
- optimising the leader producer cell line cultivation process;
- transfer of the producer cell line to production and subsequent process scale-up.

Determination of selective agent concentration for producer cell lines

Selection of Geneticin and Hygromycin concentration for CHO cell selection is shown in *Figure S1* (published on the journal website[2]). The optimal antibiotic concentrations for selecting denosumab-producing cells were shown to be 600 mg/L for Hygromycin and 600 mg/L for Geneticin. At these concentrations, cell viability reached zero by Day 7 of cultivation.

Obtaining producer minipool of denosumab recombinant protein based on CHO cell line

Transfection of CHO cells. Viable cell density (VCD) and cell viability were monitored at 24 and 48 hours post- transfection of CHO cells to assess cell survival *(Table 1)*. 48 h after transfection, the CHO cells recovered their viability, reaching a level of approximately 90%.

Obtaining producer minipools. A population of transfected cells was separated into smaller minipools and cultivated on 96-well plates with a selective agent (a total of 20 plates). The plates were incubated in a steady state in a CO_2 incubator until 70–90% confluent was achieved in BCD culture medium with selective antibiotics (600 mg/L Hygromycin and 600 mg/L Geneticin); the optimal concentration was selected in advance *(Figure S1)*. Of 1,920 minipools, 1,041 minipools have been selected (about 54% of the sample).

Screening and selecting leader minipools. On Day 10 of incubation, culture fluid samples from minipool producer cells in 96-well plates were screened using ELISA method *(Figure 2A)*. The maximum performance of minipools reached 200 mg/L; after that, the 240 highest-producing minipools were moved to 24-well plates for further use. The minipools showed a maximum productivity of 200 mg/L. Later, the 240 minipools exhibiting the highest performance (leader minipools) were chosen and transferred to 24-well plates for further experiments. Culture fluid (CF) screening of minipools from 24-well plates *(Figure 2B)* showed that the maximum productivity was 90 mg/L. Then, 88 leader minipools were transferred from 24-well plates to 6-well plates and cultivated in a steady state. Performance analysis of minipools from 6-well plates *(Figure 2C)* detected 23 leader minipools, later adapted to suspension culture with stirring in the minibioreactors in order to adapt growth and recovery of viable cells.

Thus, after several screening stages, out of 1,041 minipools, 23 minipools with the highest denosumab yield were identified through several screening rounds. These minipools were transferred to shaker culture conditions for growth adaptation and followed by cryopreservation.

Productivity and growth of the minipools. To identify the most promising candidates, the minipool producers of denosumab were cultured in a batch process for 7 days *(Figure 3)*. All the minipools maintained a high viability of ⩾90% *(Figure 3A)*. The maximum VCD (more than $\geqslant 30\times10^6$ cells/mL) was observed for 7 days for mp-14 (36.3×10^6 cells/mL), mp-16

[2] https://doi.org/10.30895/2221-996X-2025-553-fig-s1

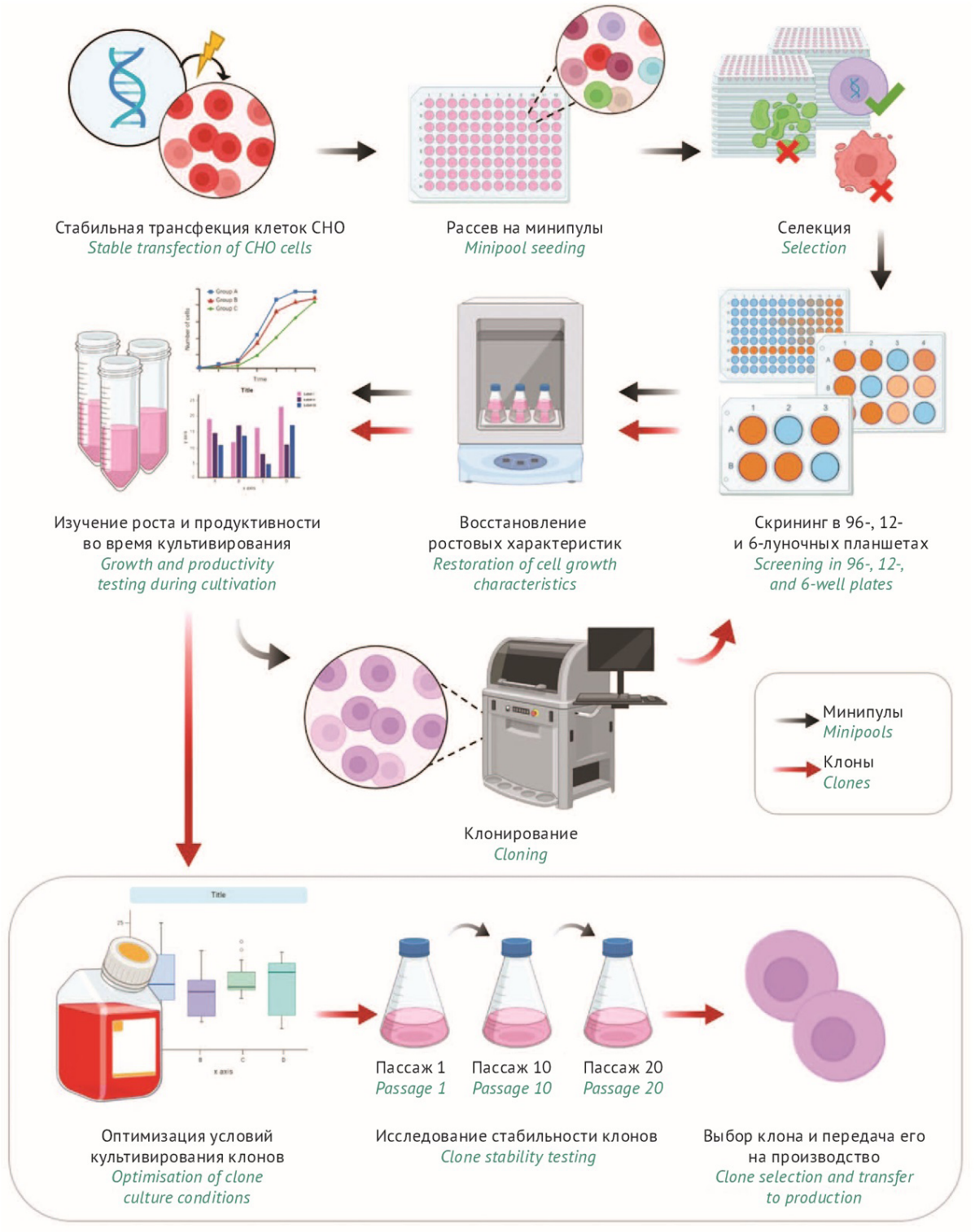

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Fig. 1. Schematic representation of obtaining CHO-based cell lines producing recombinant proteins (based on the example of denosumab).
Рис. 1. Схема получения клеточных линий-продуцентов рекомбинантного белка (на примере деносумаба) на основе клеточной линии CHO.

(34.5×10^6 cells/mL), and mp-20 (33.8×10^6 cells/mL) minipools *(Figure 3B)*. Volumetric productivity (VCD) varied from 0.6 to 1.9 g/L among the producer cell lines *(Figure 3C)*. Three minipools were identified with the highest target protein yield (≥1.7 g/L) on Day 7 during batch culture: mp-19 (1.92 g/L), mp-14 (1.82 g/L), and mp-4 (1.75 g/L).

Thus, the authors have found specific minipools of cells that showed both high cell density and the highest possible yield of the target product denosumab: mp-14, mp-4, and mp-19 *(Figure 3D)*. The producer minipool mp-19 was selected for further cloning based on a comparative analysis of physicochemical properties with the reference drug, including glycosylation profiles (results not shown).

Cloning single-cell producers of recombinant denosumab protein

Monoclonal cell lines producing denosumab were generated by seeding the leader minipool mp-19 into a semi-solid medium. Subsequently, a total of 1,056 colonies were isolated using the ClonePix FL robotic system and transferred to 12- and 96-well plates. The resulting denosumab-producing clones were successively screened via ELISA (culture fluid) to identify those with the highest productivity(specific data not shown), following the strategy outlined in *Figure 1*. Based on this screening, 24 lead producer clones were selected, adapted to shaker flask culture, and cryopreserved.

Optimising cultivation of denosumab-producing clones

Denosumab-producing clones were cultured for nine days in a continuous fed-batch process for nine days to study their growth and production characteristics (*Figure S2*, published on the journal's website[3]). The clones retained >80% viability by Day 9 *(Figure S2 A)*. The maximum VCD (>45×10^6 cells/mL) was reached on Days 6–8 of cultivation (>45×10^6 cells/mL) for the following producer clones: clone-12 (48.5×10^6 cells/mL) and clone-23 (47.7×10^6 cells/mL) *(Figure S2 B)*. Clone productivity ranged from 0.9 to 6.5 g/L *(Figure S2 C)*. Leader clones with denosumab productivity >6 g/L were identified: clone-8 (6.58 g/L), clone-13 (6.52 g/L), and clone-19 (6.04 g/L). Clones were identified that demonstrated both high VCD and product yield: clone-13, clone-8, clone-11, and clone-6 *(Figure S2 D)*. Maximum specific productivity of cell line (above 35 pg/cell/day) was shown for the following cell lines: clone-16 (38.5 pg/cell/day), clone-5 (36.2 pg/cell/day), and clone-19 (35.9 pg/cell/day) *(Table 2)*.

Thus, the authors have successfully developed high-yield monoclonal cell lines for the denosumab production, a recombinant therapeutic protein. Due to optimal parameters, a group of leader monoclonal cell lines (6–8 clones) were selected for further testing to determine whether their growth and production properties remain consistent over time.

DISCUSSION

The choice of producer cell line is critical for the development of recombinant therapeutic proteins. CHO cell line is a common choice for large-scale production of numerous recombinant proteins [19]. The experience of drug production based on this cell line can significantly reduce time and expenditures [20]. CHO cells are suitable for large-scale industrial suspension cultures, since they are able to provide high VCD and maximum protein yield [21–23]. A glycosylation profile of the produced proteins is similar to the human one, an important profit that enhances the likelihood of maintained biological activity for recombinant proteins in the human body and makes the therapy effective [24]. CHO cells have a low susceptibility to human viruses, partly due to the lacking expression of certain human viral genes, making this cell line a common choice of biological safety in large-scale drug production [25–27]. This reduced susceptibility is a key biosafety advantage, as it limits the risk of viral contamination in the production of biopharmaceuticals. The adaptability of CHO cells to pH, temperature, and oxygen fluctuations [28] allows optimising the final product quality. CHO cells can grow in serum-free media, which reduces the risk of viral and prion contamination, thereby ensuring biosafety of the recombinant proteins. The use of serum-free media also ensures

[3] https://doi.org/10.30895/2221-996X-2025-553-fig-s2

Table 1. Viability and viable cell density of CHO cells after transfection
Таблица 1. Жизнеспособность и концентрация жизнеспособных клеток CHO после трансфекции

Plasmids *Плазмиды*	Time after transfection, h *Время после трансфекции*			
	24		48	
	VCD, ×10⁶ cells/mL *VCD, ×10⁶ клеток/мл*	Viability, % *Жизнеспособность, %*	VCD, ×10⁶ cells/mL *VCD, ×10⁶ клеток/мл*	Viability, % *Жизнеспособность, %*
pGNR-FC + pGNR-FAB	1,1	68	1,83	89
Control	1,2	95	1,55	92

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Note. VCD, viable cell density.
Примечание. VCD — концентрация жизнеспособных клеток.

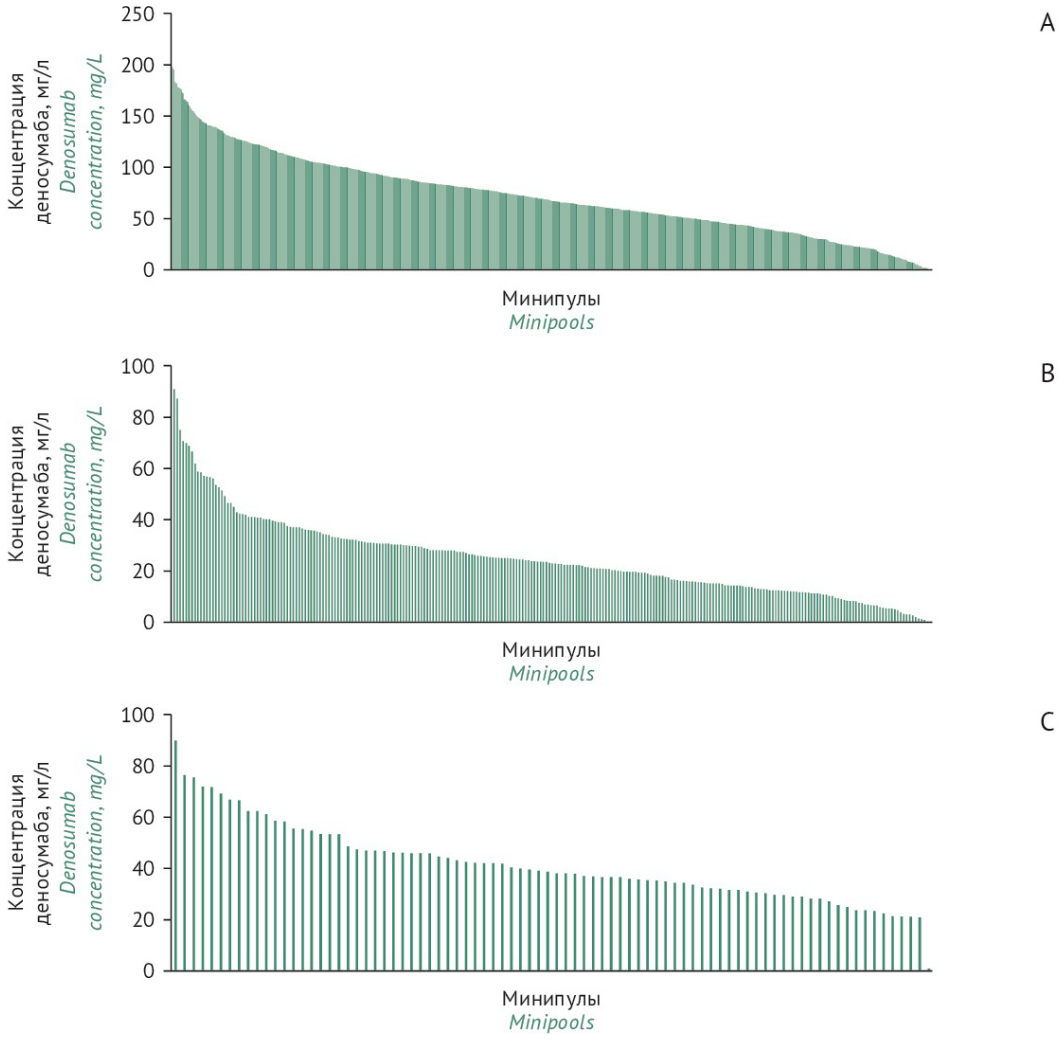

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Fig. 2. Distribution of producer minipools according to the content of the target protein (denosumab) in culture fluid during plate culture: A, 96-well plates (1041 minipools); B, 24-well plates (240 minipools); C, 6-well plates (88 minipools).
Рис. 2. Распределение минипулов-продуцентов по концентрации целевого белка (деносумаб) в культуральной жидкости при культивировании в планшетах: А – 96-луночные планшеты (1041 минипул); В – 24-луночные планшеты (240 минипулов); С – 6-луночные планшеты (88 минипулов).

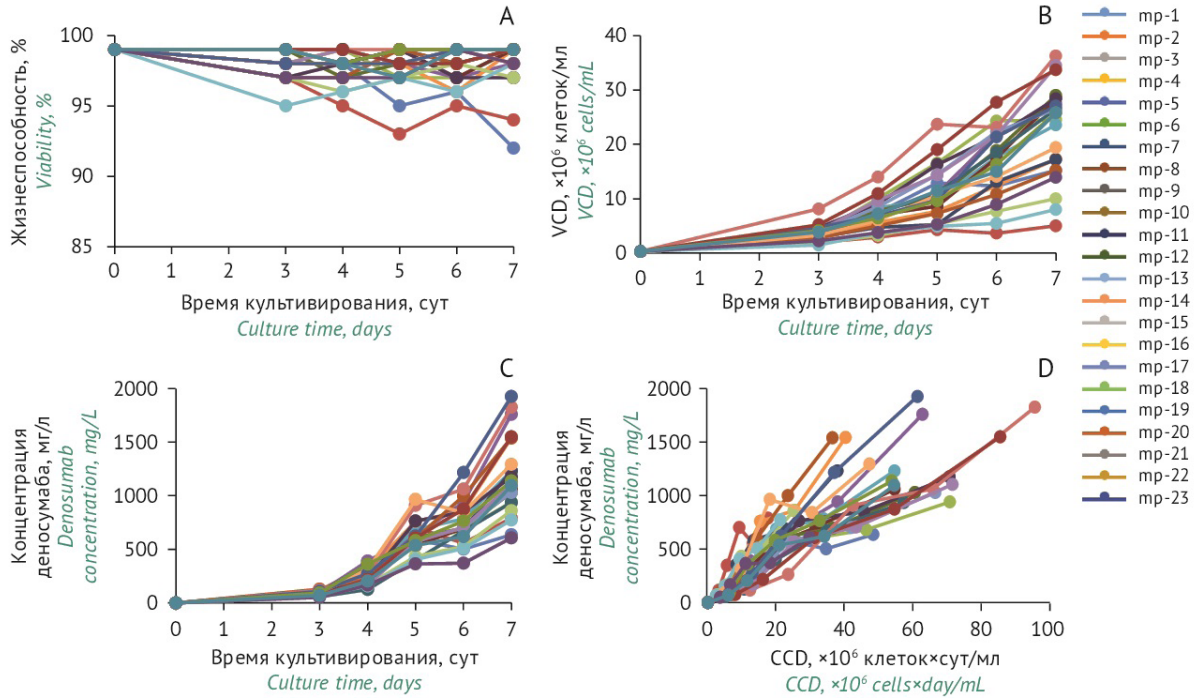
reproducibility of the protein quality profile, enhanced cell line productivity, and the optimised protein isolation and purification stages (by reducing protein impurities) [29].

A disadvantage of using CHO cells is that they can add non-human glycans, such as galactose-(alpha)-1,3-galactose and N-glycolylneuraminic acid (Neu5Gc), to therapeutic proteins [30, 31]. These glycans can increase immunogenicity and alter pharmacokinetics of the therapeutic protein [32].

The main development stages of stable monoclonal cell lines producing recombinant proteins (cell line development) can vary depending on the use of modified cell lines, expression vectors, methods, and equipment.

An expression vector should include all required regulatory elements to efficiently express a target gene integral for a parental cell line. The expression vector is selected for a specific cell type and target protein. Production of the target protein depends on a proper genetically engineered construction and the content of regulatory elements. Currently, various regulatory genetic elements that contribute to an increased yield of the target protein have been studied and approved [33].

The type of selective marker is essential in obtaining the producer line. Vectors containing antibiotic resistance genes for positive selection are traditionally used to select producers; among them is puromycin-N-acetyltransferase gene that promotes the growth of transfected cells in the presence of an antibiotic. The choice of selection system depends on the specific cell line; for instance, some lines rely on auxotrophy mechanisms, such as CHO-DG44 [34], CHO-GS, etc. Recently, CRISPR/Cas9 has been actively used to generate new cell lines and improve cellular metabolism [35].



The figure was prepared by the authors using their own data / Рисунок подготовлен авторами по собственным данным

Fig. 3. Characteristics of denosumab-producing minipools during 7 days of culture: A, cell viability; B, viable cell density (VCD); C, cell productivity; D, cumulative cell density (CCD) vs cell productivity; mp, minipools of denosumab-producing cell lines.

Рис. 3. Характеристика минипулов-продуцентов деносумаба во время культивирования в течение 7 сут: A – жизнеспособность клеток; B – концентрация жизнеспособных клеток (VCD); C – продуктивность клеток; D – зависимость кумулятивной клеточной плотности (CCD) от продуктивности. mp — минипулы-продуценты деносумаба.

Various reagents (lipofectamine, polyethyleneimine), as well as electroporators, etc., are used for transfection of eukaryotic cell lines. Electroporation is the most common method for stable transfection of eukaryotic cells [36]. It works by applying an electrical pulse to create temporary pores in the cell membrane, allowing nucleic acids to enter. Electroporation parameters (voltage, pulse and frequency duration, etc.) are specifically chosen for each cell line to optimise transfection efficiency and stable integration of genetically engineered constructs into the host cell genome.

Using a monoclonal cell line for recombinant drug based on therapeutic protein is a preferable regulatory requirement for registration. This monoclonality is crucial for a high-quality biotechnological production process using recombinant DNA technology, as well as a stable production process [37]. Monoclonal cell line development uses traditional methods such as limiting dilution, alongside special-purpose equipment used to produce a cell line from one progenitor cell with a high probability (single-cell dispensers, cell sorters, photo documentation systems, CLD cloning robotic systems, etc.). This study used ClonePix robotic system to obtain denosumab-producing cell lines by automatically selecting cell colonies from semi-solid aseptic media [38].

Developing a highly productive cell line requires a large number of cell lines. In this research, 1,041 minipools (approximately 54%) were selected from a total of 1,920. Of these, 4 minipools with productivity exceeding 1.5 g/L (0.2%) were identified during the batch cultivation for 7 days. The leader minipool with a productivity of about 2 g/L was then cloned, resulting in 1,056 monoclonal cell lines. Three monoclones with a productivity ≥6 g/L (0.3%) were identified during cultivation. Thus, monoclones were found based on the existing sample of minipools and clones; these monoclones provide a target protein titre of more than 6 g/L for 9 days. This approach helps predict the amount of cell lines required to achieve the target protein yield. Currently, mathematical modeling is frequently used to select the most preferred cell lines by various parameters [39].

Developing monoclonal producer cell lines can vary significantly depending on the choice of host cell line, expression vectors, materials, assay methods, and equipment at various development stages. Achieving high titres of target recombinant protein became possible due to complex measures focused on optimising the expression system; selecting

Table 2. Specific productivity of clones producing denosumab
Таблица 2. Специфическая продуктивность клонов-продуцентов деносумаба

Clone *Клон*	Specific performance, pg/cell/day *Специфическая продуктивность, пг/клетка/сут*				
	Culture time, days *Время культивирования, сут*				
	5	6	7	8	9
clone-1	11,6	14,4	5,4	23,0	16,9
clone-2	14,7	28,4	13,1	29,5	26,4
clone-3	2,8	8,8	9,8	10,2	17,6
clone-4	8,7	22,1	20,2	22,5	15,8
clone-5	11,3	27,5	14,6	26,1	36,2
clone-6	9,7	14,5	18,2	21,1	24,9
clone-7	10,6	18,3	14,6	17,7	22,6
clone-8	14,3	18,9	8,1	24,0	32,2
clone-9	8,5	16,1	9,1	22,6	19,7
clone-10	14,3	20,5	36,9	22,1	13,1
clone-11	9,1	16,8	6,6	13,5	28,4
clone-12	6,0	15,1	15,6	14,4	11,1
clone-13	12,6	23,0	14,0	20,7	32,5
clone-14	4,9	15,2	24,8	16,3	25,0
clone-15	10,7	19,2	20,4	21,9	31,9
clone-16	8,4	18,6	19,6	22,0	38,5
clone-17	0,0	5,2	13,7	13,7	11,2
clone-18	8,0	14,1	14,9	17,6	27,2
clone-19	8,9	18,4	16,5	21,5	35,9
clone-20	0,1	6,8	20,1	10,3	5,0
clone-21	5,6	13,4	16,1	12,0	20,3
clone-22	0,0	9,1	28,9	11,2	18,6
clone-23	3,2	9,2	17,5	9,5	19,2
clone-24	2,0	5,5	28,1	15,1	20,6

The table was prepared by the authors using their own data / Таблица составлена авторами по собственным данным

Note. Clone, monoclonal cell lines producing denosumab.
Примечание. Clone – моноклональные клеточные линии-продуценты деносумаба.

host cells, screening methods, producers, and high-technology equipment; optimising the growth media and feedstocks with certain chemical composition; and using innovative solutions in the area.

The growing demand for biotherapeutic products, particularly in chronic diseases like cancer/autoimmune/orphan disorders, creates a pressing need to shorten the time and lower the cost of cell line development.

This results in improved methods, reagents, and equipment used to obtain producer cell lines.

The authors have rendered their approach and described main development stages of stable monoclonal cell lines producing recombinant proteins based on suspension-culture CHO cell line, using denosumab producers as the case.

CONCLUSIONS

1. A process was developed to obtain highly productive cell line recombinant proteins based on CHO suspension cell line.
2. High-yield monoclonal cell lines producing denosumab were obtained with a yield of more than 6 g/L.
3. Our approach to creating producer cell lines can be used to develop commercially significant recombinant proteins, including monoclonal antibodies, enzymes, blood coagulation factors, and other proteins.

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