Improved cultivation of CHO cells in bioreactor with reciprocal mixing

Masashi UEKI[1], Noriyuki Tansho[2], Makoto Sato[2], Hisayuki Kanamori[2], Yoshihiro Ito[1], and Yoshikazu Kato[2]

[1]RIKEN
[2]Satake Chemical Equipment Mfg., Ltd., Japan

July 29, 2020

Abstract

We have constructed a new bioreactor with reciprocal mixing that is better suited for the cultivation of delicate animal cells. In-silico simulation (computational fluid dynamics) suggested both maximum and average shear stresses in the bioreactor with reciprocal mixing to be remarkably lower than in conventional bioreactor with rotary mixing. Although we could not find any difference in growth speed and cell density between the bioreactors with reciprocal and rotary mixing, we did find cell viability in reciprocal-mixing bioreactor to be retained longer than in rotary-paddle bioreactor. This implied that cell culture in a bioreactor with reciprocal mixing could be prolonged for the production of target proteins. Leakage of lactate dehydrogenase activity into the culture medium was suppressed much more in the reciprocal-mixing bioreactor than in the rotary-paddle one. Production of human tissue plasminogen activator in the former was also observed to be much more than in the latter. Therefore, bioreactor with reciprocal mixing was concluded to be better suited for the cultivation of animal cells and efficient production of proteins, such as antibody drugs and various growth factors.

1. Introduction

Cell culture environment, which includes homogeneity of nutrients and metabolic wastes, as well as exchange efficiency of dissolved gases, significantly affects cell growth and target protein productivity. Although higher mixing speed might maintain homogeneity and efficiency in cell culture, it generates a destructive shear stress on the cells. Classical small-scale culture systems, such as planar apparatus (e.g., culture plates and T-flasks) or shaker flasks (e.g., Erlenmeyer flasks), have several drawbacks regarding cell production yield, with limited scalability, and lack of control on culture parameters (Birch, 1990; Steiner, 2010; Abecasis, 2017). Various bioreactor designs are currently available for cell culture, namely the perfusion bioreactor (Serra, 2010; Simao, 2016; Bancroft, 2003), rotating wall bioreactor (Radtke, 2012; Navran, 2008), and spinner flask bioreactor (Ismadi, 2014; Gupta, 2016). Optimizing the culture conditions in a bioreactor has been an important technical factor to achieve large cell numbers as required for therapeutic purposes (Serra, 2012; Abecasis, 2017; Martin, 2004; Kropp, 2016). The spinner flask bioreactor is the most commonly used type for suspension culture owing to its easy setup and availability. However, there are some difficulties in the design and setup of a generic bioreactor for a wide range of cell types.

Turning or rotary paddles, which enable quick exchange of dissolved air, is also a common suspension culture system. Faster stirring improves the efficiency of mixing, although the shear stress increases, resulting in cell damage (Rawat, 2016). However, if stirring is slowed down, the efficiency of mixing decreases, and uniformity of the culture medium may be lost. Optimal culture conditions are required for balancing the outcome. For large-scale industrial production, energy saving features need to be considered in addition, based on stirring power.

A culture bioreactor, with a new stirring concept (reciprocal motion), has been developed recently (Fig. 1). The culture medium is stirred by an elliptic horizontal plate moving up and down. According to the scale of culture, one elliptic plate, or two elliptic plates with a 90-degree shift may be installed in the bioreactor. For a bioreactor, sealing is an important consideration. A rubber board or bellow-like pipe was designed to seal the bioreactor with reciprocal mixing (Fig.1C). In this report, we aimed to discuss the availability and advantages of the new-concept bioreactor for animal cell culture, comparing it with the conventional bioreactor with rotary paddles.

2. Materials and methods

2.1. Cell lines and media

Chinese hamster ovary cells for suspension culture (CHO-S) were purchased from Gibco, USA. CHO 1-15 cells (CRL-9606) were purchased from ATCC, USA. Ham's F-12 (Wako Pure Chemical Corporation, Japan) with 10% FBS (Thermo Fisher Scientific, USA) and CHO-S-SFM II (Thermo Fisher Scientific, USA) were used for culture of CHO-S and CHO 1-15 cells, respectively. All media were supplemented with penicillin/streptomycin solution (Nacalai Tesque, Japan). Cells were maintained in CO_2 incubator (Panasonic, Japan), at 37 °C with air containing 5% CO_2. Cell number was measured by Cell Counter TC20 (Bio-Rad, USA) after staining cells with 0.5% trypan blue solution (Nacalai Tesque, Japan).

2.2. Cultivation in bioreactor

Overall structure of bioreactor used in this work is shown in Fig. 1. DO sensor and pH electrode (Millipore) were installed in the vessel. A 3.0-L glass vessel containing 2.4 L of medium was used for starting batch culture. As shown in detail in Fig. 1B–D, a double-elliptic plate (RM) was used for reciprocal mixing (stroke = 20 mm), and conventional 6 flat turbine (6FT) and elephant ear impeller (EE) were used for rotary mixing. Cells, prepared in 100-mm dishes and 100-mL flask with shaking, were transferred into the bioreactor to start cultivation at 10^5 cells/mL. Bioreactors were operated at Pv (power per unit volume) 36 W/m^3, which is equivalent to 135 rpm for 6FT mixing, 140 rpm for EE mixing, and 80 mm/s (= 2.0 Hz) for reciprocal mixing.

2.3. Measurement of contents in medium

An aliquot of culture broth was withdrawn to check cell number and viability, and to measure the amount of nutrient remaining and amount of metabolites produced. Cell number and viability were measured by Cell Counter (Bio-Rad). Residual glucose and production of lactate, glutamine, glutamate, and ammonium salt in sampled broth were measured by multifunctional biosensor BF-7D (Oji Scientific Instruments, Japan). LDH activity was measured by LDH assay kit (Wako Chemical, Japan) according to the manufacturer's instruction. Activity of human tissue plasminogen activator (tPA) was measured by colorimetric method using S-2288 (Diapharma, USA), as described previously (Feng, 1995).

2.4. Computational fluid dynamics (CFD) simulation

Computational fluid dynamics simulation software Fluent (ANSYS, USA) was used for the calculations of shear stress and vectors. Rotary bioreactors (rotary-paddle bioreactors) were used with steady-state analysis and realizable κ-ϵ model, and reciprocal bioreactors (reciprocal-mixing bioreactors) were used with non-steady-state analysis and realizable κ -ϵ model. Physical properties, used in the CFD simulations of CHO cell culture, were set as measured values of viscosity (5 mPa-s) and specific density (1005 kg/m^3).

3. Results

3.1. Cultivation of CHO-S cells in bioreactor Growth curves and viability are shown in Fig. 2. Growth rate during log phase in a bioreactor with reciprocal mixing was not significantly different from that in a bioreactor with rotary mixing, namely 6FT or EE. Cell density in all bioreactors reached approximately 6.0×10^6 cells/mL, with no significant difference across them all (Fig. 2B). This implied that the mixing methods (rotary or reciprocal) did not affect the growth rate during log phase or the cell density achieved after exponential growth. However, the length of stationary phase in reciprocal bioreactor was longer than

that in both the rotary bioreactors. Viability in 6FT and EE rotary bioreactors went to less than 90% (˜ 130 h), faster than that in reciprocal bioreactor (160 h, Fig. 2A). Concentrations of glucose, lactate, and leaked lactate dehydrogenase activity in culture medium are shown in Fig. 3. Significant difference in glucose consumption was not observed in any bioreactor (Fig. 3A). In our cases of batch culture, consumption rate of lactate was more than its production rate when cell growth entered the stationary phase (at approximately 80 h, Fig. 3B). However, the concentration of lactate in reciprocal bioreactor decreased faster than in other rotary bioreactors. This revealed that physiological conditions of cells in all bioreactors proceeded from the production to consumption of lactate in approximately 80 h, and at the same time something else also changed that caused the difference in viability during the late stationary phase. High concentration of lactate in culture medium is detrimental for viable cells. Therefore, the rapid consumption of lactate in reciprocal bioreactor might make conditions more feasible for maintaining high cell viability.

3.2. Leakage of lactate dehydrogenase activity

Faster stirring improves the efficiency of mixing, although it simultaneously increases the shear stress, eventually causing cell damage. Animal cells, which do not have cell walls, are fragile to physical stress. To check the damage during cell culture, we studied the leakage of lactate dehydrogenase (LDH) activity in the culture medium. As shown in Fig. 3C, in the bioreactor with 6FT or EE, leaked LDH activity rapidly increased after 120 h while reciprocal mixing had suppressed leakage. If the leaked LDH activity, during rotary mixing with EE, has to be suppressed as low as that in reciprocal mixing, stirring speed should be decreased to less than 100 rpm (data not shown). Stirring with EE at 100 rpm would induce insufficient mixing, resulting in poor growth and unsatisfactory gas exchange.

3.3. CFD analysis

Differences were observed between the culture results of the rotary bioreactor and reciprocal bioreactor. The differences seemed to be influenced by the physical properties of each bioreactor. CFD simulations were used to clarify the relationship between culture results and physical action. In general, low shear stress is required for animal cell culture; however, our study showed uniform floating and dispersal of cells, and appropriate stimulus to cells to also be important.

Fig. 4A shows the vector of rotary bioreactor with EE, and Fig. 4B–D show the vector at the impeller positions “top dead point”, “passing through middle point”, and “bottom dead point”, respectively, of a reciprocal bioreactor. In the rotary bioreactor, weak flow zone exists near the center, under the impeller; however, in the reciprocal bioreactor, flow pattern spreads throughout the entire culture vessel. Such flow, as in the reciprocal bioreactor, is very important for cell culture. Fig. 4E shows the shear stress in rotary bioreactor, and Fig. 4F–H show the shear stress at the impeller positions, as shown above. Although the most abundant shear stress (around 1 Pa, Fig. 5) was similar to each other, characteristic distribution curve possessing two peaks was drawn in reciprocal bioreactor. Most importantly, the stress generated by reciprocal motion was not continuous while that in rotary bioreactor was generated continuously. Industrial production using animal cell culture starts by switching to protein production when cell growth reaches a stationary state. Therefore, the damage that cells undergo while facing prolonged and continuous shear stress in rotary bioreactor is considered to be more serious than that caused by discontinuous shear stress in reciprocal bioreactor. Moreover, it is also undesirable for the cell environment, since shear stress creates in a non-uniform condition in the bioreactor.

As shown in Table 1, both maximum and average shear stresses in rotary bioreactor were found to be higher than that in the reciprocal bioreactor. In addition, the simulation showed a large variation in the shear stress and shear rate in the rotary bioreactor. Cells in rotary bioreactor are continuously exposed to such a stressful turbulence, and accumulate the damage to eventually affect their physiological condition. Thus, the physical characteristics of the reactor are reflected in the culture results.

3.4. Production of human tissue plasminogen activator (tPA) by CHO 1-15 cells

We next examined whether reciprocal mixing affects the production of a target protein. In this study, we

compared the production of tPA by CHO 1-15 cells in EE and RM bioreactors, operated at Pv = 36 W/m^3 (equivalent to 2.0 Hz (80 mm/s) for reciprocal, and 140 rpm for EE rotary). Activity of tPA increased during exponential phase in both the bioreactors, along with increasing cell number (Figs. 6, 7). After passing through the late exponential or early stationary phase (at approximately 100–120 h), CHO 1-15 cells in reciprocal bioreactor were found to produce tPA activity 1.3–1.5 times higher than in EE bioreactor (Fig. 7). It implied that the total amount of tPA protein produced in reciprocal bioreactor was approximately 1.5 times as much as in EE bioreactor. Moreover, cell viability in EE bioreactor rapidly decreased after approximately 200 h (Fig. 6A). The culture medium in EE bioreactor was found to contain undesirable impurity, such as cell debris derived from destroyed or dead cells. Therefore, some definitive advantages do exist in reciprocal-mixing bioreactor.

4. Discussion

Although a bioreactor with reciprocal mixing cannot be simply compared to conventional bioreactors with rotary paddles, the cultivation conditions of CHO cells in both bioreactors, in terms of cell growth, damage, and lactate production were compared in this report. There was no significant difference between the bioreactors in terms of cell proliferation and density during exponential phase. However, in the reciprocal bioreactor, the transition from stationary to death phase was slower, and high cell viability was maintained for a longer period than in the bioreactor with rotary paddles. Moreover, lactate dehydrogenase (LDH) activity in the medium of reciprocal bioreactor was much lower than in the rotary bioreactor. Since LDH is a cell damage marker, shear stress on cells in the reciprocal bioreactor was shown to be overall lower than in the rotary bioreactor. The condition of cells, after entering early stationary phase, is important for industrial production of targeted proteins. Therefore, maintaining high cell viability after stationary phase is critical for high-quality production.

When shear stress in bioreactors was analyzed by CFD, strong shear stress generated by separation vortex was observed around the rotary paddles, and cells were found to be exposed to it throughout the cultivation process. On the other hand, maximum shear stress in reciprocal mixing was observed around the plates when they turned at the top and bottom dead points. Shear stress in reciprocal mixing was minimized when the plates passed through the middle point of stroke. This non-steady-state mixing of reciprocal motion could dramatically reduce the accumulation of cell damage, thereby affecting the physiological conditions of living cells.

Influence of shear stress on cell damage has been investigated not only in bioreactors, but also in microfluid devices and blood vessels (Mitchell, 2013; Odeleye, 2014; Model, 2014). Although many researchers have tried to clarify how shear stress affects gene expression and physiological conditions (Nurhayati, 2018; Jayagopal, 2019), the kind of cellular systems that sense the effect of shear stress on gene expression still remains unknown. Even if a type of shear stress induced gene expression in one cell type, it may not affect the same in other cell lines (Akimoto, 2000; Novak, 2019). Therefore, how reciprocal mixing could influence gene expression and intracellular physiological conditions, resulting in the maintenance of high cell viability and target protein expression, needs to be clarified in future.

Recently, Eto's group reported a bioreactor with reciprocal mixing to be effective for platelet generation from human iPS-derived megakaryocytes (Ito, 2018). Platelet biogenesis from megakaryocytes requires blood flow-dependent shear stress (Junt, 2008). While other bioreactors with rotary paddles or culture bags could not produce sufficient and appropriate levels of shear stress, a bioreactor with reciprocal motion could generate proper turbulent energy in the culture medium, thereby inducing in-vitro thrombopoiesis.

Development of a bioreactor, till date, has mainly focused on the growth of cultured organism, production of targeted compounds or proteins, and homogeneity of culture broth. A wide variety of ideas, including different kinds of mixing impellers, and rotating or see-sawing culture vessels have been developed over the years (Birch, 1990; Rotenberg, 2012). However, mixing and dispersing actions are inversely proportional to shear stress, in case of conventional bioreactors, making it difficult to optimize the culture conditions (Wyma, 2018). In this study, we have introduced a novel concept of bioreactors for culturing animal cells,

and clarified the specific characteristics of a bioreactor with reciprocal mixing in comparison to those of a conventional bioreactor with rotary paddles. Although the reason behind the appreciable effect of reciprocal mixing on cell growth remains unknown, shear stress generated by reciprocal motion might be physiologically acceptable for cell growth. Studies are ongoing for understanding the processes occurring in cells when they are exposed to reciprocal shear stress.

Acknowledgement

This work was partially supported by RIKEN's Integrated Collaborative Research Program with Industry.

References

Abecasis, B., Aguiar, T., Arnault, É., Costa, R., Gomes-Alves, P., Aspegren, A., Serra, M., & Alves, P. M. (2017). Expansion of 3D human induced pluripotent stem cell aggregates in bioreactors: Bioprocess intensification and scaling-up approaches. *Journal of Biotechnology* , *246* , 81-93. doi: 10.1016/j.jbiotec.2017.01.004.

Akimoto, S., Mitsumata, M., Sasaguri, T., & Yoshida, Y. (2000). Laminar shear stress inhibits vascular endothelial cell proliferation by inducing cyclin-dependent kinase inhibitor p21(Sdi1/Cip1/Waf1). *Circulation Research* , *86* , 185-190. doi: 10.1161/01.res.86.2.185.

Bancroft, G. N., Sikavitsas, V. I., & Mikos, A. G. (2003). Design of a flow perfusion bioreactor system for bone tissue-engineering applications. *Tissue Engineering, 9,* 549-554. doi: 10.1089/107632703322066723.

Birch, J. R. & Arathoon, R. (1990). Suspension culture of mammalian cells. *Bioprocess Technology* , *10* , 251-270.

Feng, Y. H. & Hart, G. (1995). In vitro oxidative damage to tissue-type plasminogen activator: a selective modification of the biological functions. *Cardiovascular Research, 30* , 255-261.

Gupta, P., Ismadi, M. Z., Verma, P. J., Fouras, A., Jadhav, S., Bellare, J., & Hourigan, K. (2016). Optimization of agitation speed in spinner flask for microcarrier structural integrity and expansion of induced pluripotent stem cells. *Cytotechnology, 68* , 45-59. doi: 10.1007/s10616-014-9750-z.

Ismadi, M. Z., Gupta, P., Fouras, A., Verma, P., Jadhav, S., Bellare, J., & Hourigan, K. (2014). Flow characterization of a spinner flask for induced pluripotent stem cell culture application. *PLoS One, 9,*e106493. doi: 10.1371/journal.pone.0142287.

Ito, Y., Nakamura, S., Sugimoto, N., Shigemori, T., Kato, Y., Ohno, M., ..., Eto, K. (2018). Turbulence activates platelet biogenesis to enable clinical scale ex vivo production. *Cell* , *174* , 636-648. doi: 10.1016/j.cell.2018.06.011.

Jayagopal, A., Brakeman, P. R., Soler, P., Ferrell, N., Fissell, W., Kroetz D. L., & Roy, S. (2019). Apical shear stress enhanced organic cation transport in human OCT2/MATE1-transfected madin-darby canine kidney cells involves ciliary sensing. *The Journal of Pharmacology and Experimental Therapeutics* , *369* , 523-530. doi: 10.1124/jpet.118.255026.

Junt, T., Schulze, H., Chen, Z., Massberg, S., Goerge, T., Krueger, A., ..., von Andrian U. H. (2007). Dynamic visualization of thrombopoiesis within bone marrow. *Science* , *317* , 1767-1770. doi: 10.1126/science.1146304.

Kropp, C., Kempf, H., Halloin, C., Robles-Diaz, D., Franke, A., Scheper, T., ..., Olmer, R. (2016). Impact of feeding strategies on the scalable expansion of human pluripotent stem cells in single-use stirred tank bioreactors. *Stem Cells Translational Medicine* , *5* , 1289-1301. doi: 10.5966/sctm.2015-0253.

Martin, I., Wendt, D., & Heberer, M. (2004). The role of bioreactors in tissue engineering. *Trends in Biotechnology* , *22* , 80-86. doi: 10.1016/j.tibtech.2003.12.001.

Mitchell, M. J., & King, M. R. (2013). Fluid shear stress sensitizes cancer cells to receptor-mediated apoptosis via trimeric death receptors. *New Journal of Physics* , *15* , 015008. doi: 10.1088/1367-2630/15/1/015008.

Model, L. S., Hall, M. R., Wong, D. J., Muto, A., Kondo, Y., Ziegler, K. R., ..., Dardik, A. (2014). Arterial shear stress reduces Eph-B4 expression in adult human veins. *The Yale Journal of Biology and Medicine* , *87* , 359-371.

Navran, S. (2008). The application of low shear modeled microgravity to 3-D cell biology and tissue engineering. *Biotechnology Annual Review* , *14* , 275-296. doi: 10.1016/S1387-2656(08)00011-2.

Novak, C. M., Horst, E. N., Taylor, C. C., Liu, C. Z., & Mehta, G. (2019). Fluid shear stress stimulates breast cancer cells to display invasive and chemoresistant phenotypes while upregulating PLAU in a 3D bioreactor. *Biotechnology and Bioengineering* , *116* , 3084-3097. doi: 10.1002/bit.27119.

Nurhayati, R. W., Ojima, Y., Dohda, T., & Kino-Oka, M. (2018). Large scale culture of a megakaryocytic progenitor cell line with a single-use bioreactor system. *Biotechnology Progress* , *34* , 362-369. doi: 10.1002/btpr.2595.

Odeleye, A. O. O., Marsh, D. T. J., Osborne, M. D., Lye, G. J., & Micheletti, M. (2014). On the fluid dynamics of a laboratory scale single-use stirred bioreactor. *Chemical Engineering Science* ,*111* , 299-312. doi: 10.1016/j.ces.2014.02.032.

Radtke, A. L. & Herbst-Kralovetz, M. M. (2012). Culturing and applications of rotating wall vessel bioreactor derived 3D epithelial cell models. *Journal of Visualized Experiments, 62* , 3868. doi: 10.3791/3868.

Rawat, J. & Gadgil, M. (2016). Shear stress increases cytotoxicity and reduces transfection efficiency of liposomal gene delivery to CHO-S cells. *Cytotechnology* , *68* , 2529-2538. doi: 10.1007/s10616-016-9974-1.

Rotenberg, M. Y., Ruvinov, E., Armoza, A., Cohen, S. (2012). A multi-shear perfusion bioreactor for investigating shear stress effects in endothelial cell constructs. *Lab on a Chip* , *12* , 2696-2703. doi: 10.1039/c2lc40144d.

Serra, M., Brito, C., Correia, C., & Alves, P. M. (2012). Process engineering of human pluripotent stem cells for clinical application. *Trends in Biotechnology* , *30* , 350-359. doi: 10.1016/j.tibtech.2012.03.003.

Serra, M., Brito, C., Sousa, M. F. Q., Jensen, J., Tostoes, R., Clemente, . . . , Alves, P. M. (2010). Improving expansion of pluripotent human embryonic stem cells in perfused bioreactors through oxygen control. *Journal of Biotechnology* , *148* , 208-215. doi: 10.1016/j.jbiotec.2010.06.015.

Simão, D., Arez, F., Terasso, A. P., Pinto, C., Sousa, M. F., Brito, C., & Alves, P. M. (2016). Perfusion stirred-tank bioreactors for 3D differentiation of human neural stem cells. *Methods in Molecular Biology, 1502* , 129-142. doi: 10.1007/7651_2016_333.

Steiner, D., Khaner, H., Cohen, M., Even-Ram, S., Gil, Y., Itsykson, P., . . . , Reubinoff, B. (2010). Derivation, propagation and controlled differentiation of human embryonic stem cells in suspension. *Nature Biotechnology* , *28* , 361-364. doi: 10.1038/nbt.1616.

Wyma, A., Martin-Alarcon, L., Walsh, T., Schmidt, T. A., Gates, I. D., & Kallos, M. S. (2018). Non-newtonian rheology in suspension cell cultures significantly impacts bioreactor shear stress quantification. *Biotechnology and Bioengineering* , *115* , 2101-2113. doi: 10.1002/bit.26723.

Figure legends

Fig. 1. Bioreactor system and mixing impellers used in this study.

(A) Structure of a bioreactor. A water-jacketed 2.5-L glass vessel containing 2 L of medium. Air containing 5% CO_2 was supplied at 50 mL/min onto the surface of medium; supply of pure O_2 through sparger to the inside of medium was controlled by DO sensor to maintain $>$ 2.8 mg O_2/L. (B) Six flat turbine impeller (6FT). (C) Conventional elephant ear impeller (EE). (D) Double elliptical plates for reciprocal mixing (RM).

Fig. 2. Growth curves and viabilities of CHO-S cells.

(A) Cell viabilities. (B) Growth curves. CHO-S cells were cultivated in bioreactors with 6FT (grey triangle), $EE(open rectangle), or RM(closed circle *)$. $Batch culture was started by seeding preculturedcells in the bioreactor at 10^5 cells/m$

Fig. 3. Analyses of culture media for culturing CHO-S cells.

(A) Glucose concentration in media. (B) Lactate concentration in media. (C) Leaked LDH activity. CHO-S cells were cultivated in bioreactors with 6FT (grey triangle), $EE(open rectangle), or RM(closed circle *). No significant difference was observed before 80h. After entering the early station$

Fig. 4. Computational fluid dynamics analyses at Pv = 36 W/m^3.

(A–D) Velocity vectors. (E–H) Contours of shear stress. (A, E) Rotary mixing with EE. (B–D, F–H) Reciprocal mixing with RM.

Fig. 5. Distribution of shear stress in the bioreactors.

Distribution of shear stress in bioreactors with EE (open rectangle) and RM (closed circle *). Most abundant shear stress appeared around 100 Pa in both bioreactors, and maximum stress was not so different between the two. However, shear stress in rotary bioreactor was generated continuously, as opposed to discontinuous shear stress in reciprocal bioreactor.

Fig. 6. Growth curves and cell viabilities of CHO 1-15 cells.

CHO 1-15 cells were cultivated in bioreactors with EE (open rectangle) or RM (closed circle *). (A) Cell viabilities. (B) Growth curves. Batch culture was started at 4 x 10^4 cells/mL. There was no significant difference in terms of cell growth in either bioreactor; however, cell viability in EE bioreactor suddenly decreased at 200 h.

Fig. 7. tPA activity produced by CHO 1-15 cells.

Production of tPA by CHO 1-15 cells began, along with cell growth, in the bioreactor with EE (open rectangle) and RM (closed circle *).

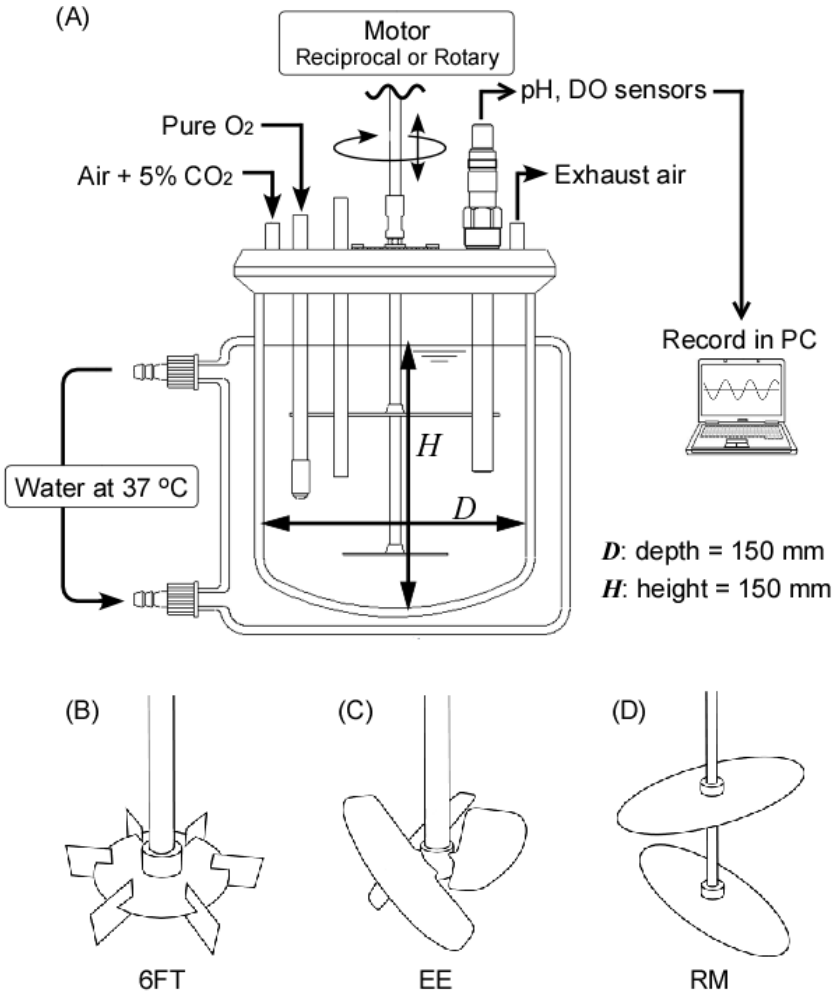

Fig. 1. Bioreactor system and mixing impellers used in this study.

(A) Structure of a bioreactor. A water-jacketed 2.5-L glass vessel containing 2 L of medium. Air containing 5% CO_2 was supplied at 100 mL/min onto the surface of medium; supply of pure O_2 through sparger to the inside of medium was controlled by DO sensor to maintain > 6.0 mg O_2/L. (B) Six flat turbine impeller (6FT). (C) Conventional elephant ear impeller (EE). (D) Double elliptical plates for reciprocal mixing (RM).

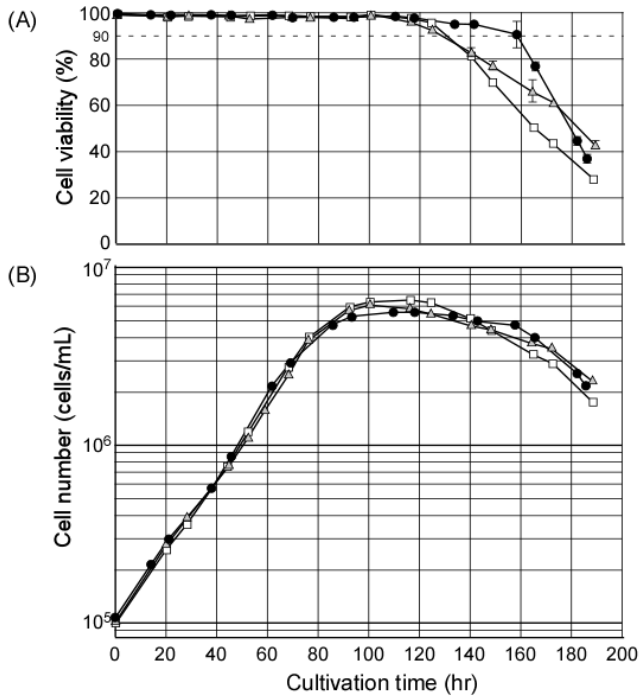

Fig. 2. Growth curves and cell viabilities of CHO-S cells.

(A) Cell viabilities. (B) Growth curves. CHO-S cells were cultivated in bioreactors with 6FT (grey triangle ▲), EE (open rectangle □), or RM (closed circle ●). Batch culture was started by seeding precultured cells in the bioreactor at 10^5 cells/mL. No significant difference in terms of cell number and cell viability was observed before 120 h. However, cell viability in 6FT and EE bioreactors rapidly decreased at around late stationary phase (120 h).

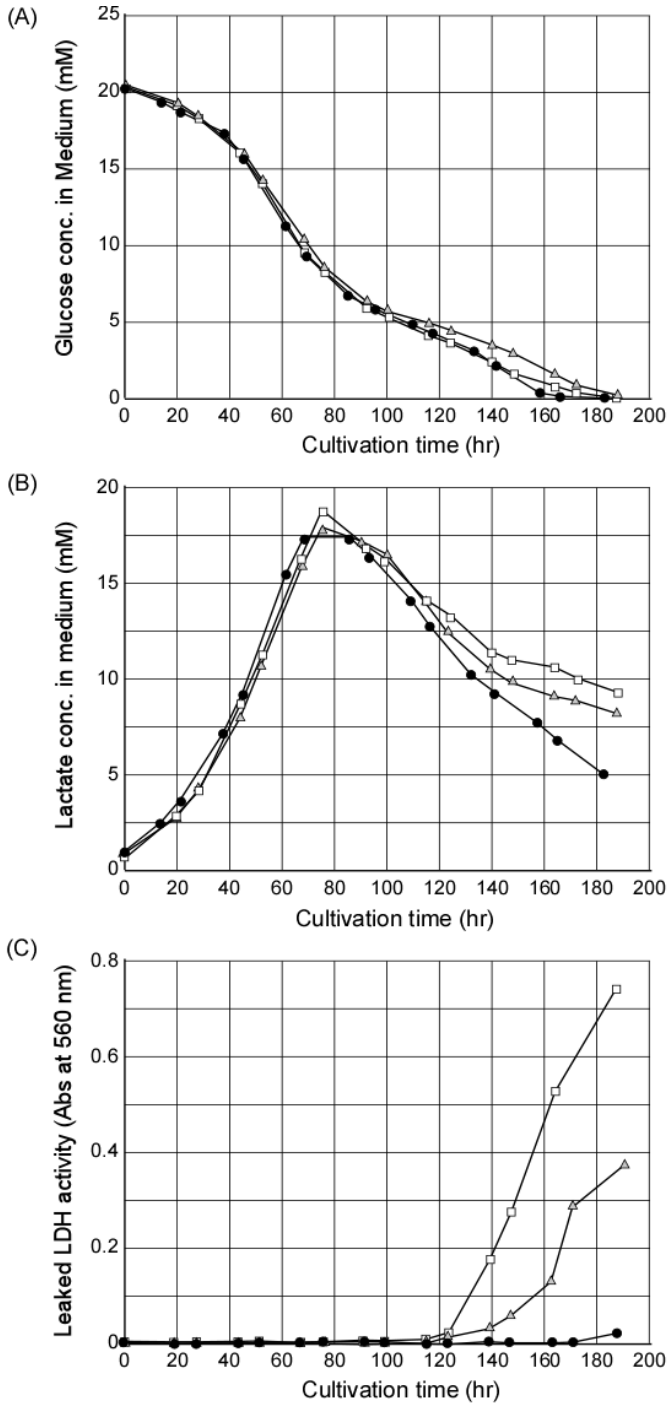

Fig. 3. Analyses of culture media culturing CHO-S cells.

(A) Glucose concentration in media. (B) Lactate concentration in media. (C) Leaked LDH activity. CHO-S cells were cultivated in bioreactors with 6FT (grey triangle ▲), EE (open rectangle □), or RM (closed circle ●). No significant difference was observed before 80 h. After entering the early stationary phase (at approximately 80 h), lactate consumption became much more than lactate production. Concentration of lactate in bioreactor with RM decreased rapidly. Leaked LDH activity in bioreactors with 6FT and EE was drastically increased after late stationary phase (approximately 160 h) while that in reciprocal bioreactor was suppressed to lower level.

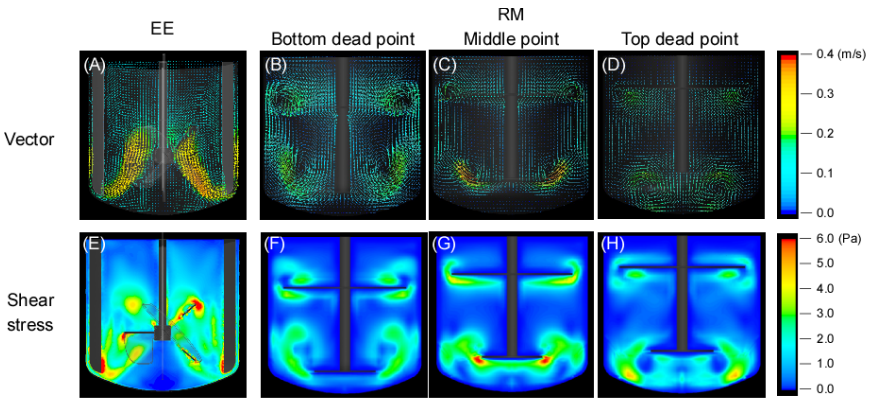

Fig. 4. Computational Fluid Dynamic Analyses at Pv = 36 W/m^3.

(A-D) Velocity vectors. (E-H) Contours of shear stress. (A, E) Rotary mixing with EE. (B-D, F-H) Reciprocal mixing with RM.

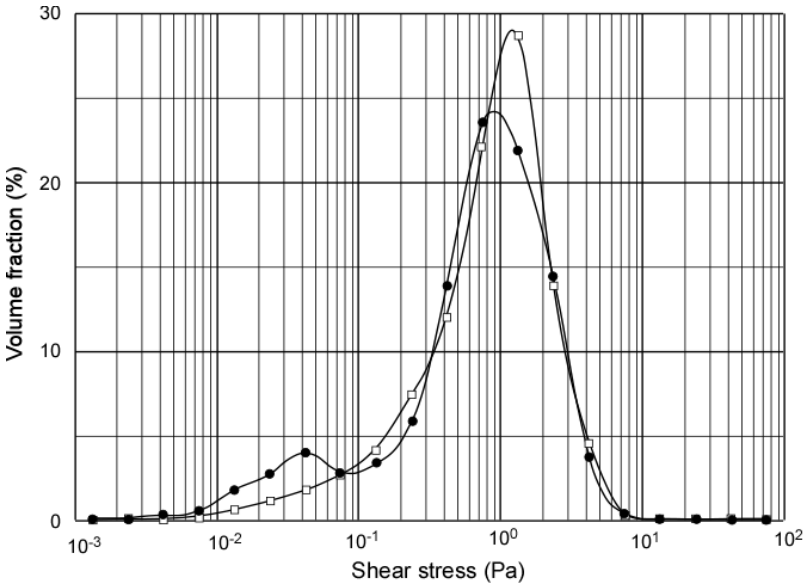

Fig. 5. Distribution of shear stress in the bioreactors.

Distribution of shear stress in bioreactors with EE (open rectangle □) and RM (closed circle ●). Most abundant shear stress appeared around 100 Pa in both bioreactors, and maximum stress was not so different between the two. However, shear stress in rotary bioreactor was generated continuously, as opposed to discontinuous shear stress in reciprocal bioreactor.

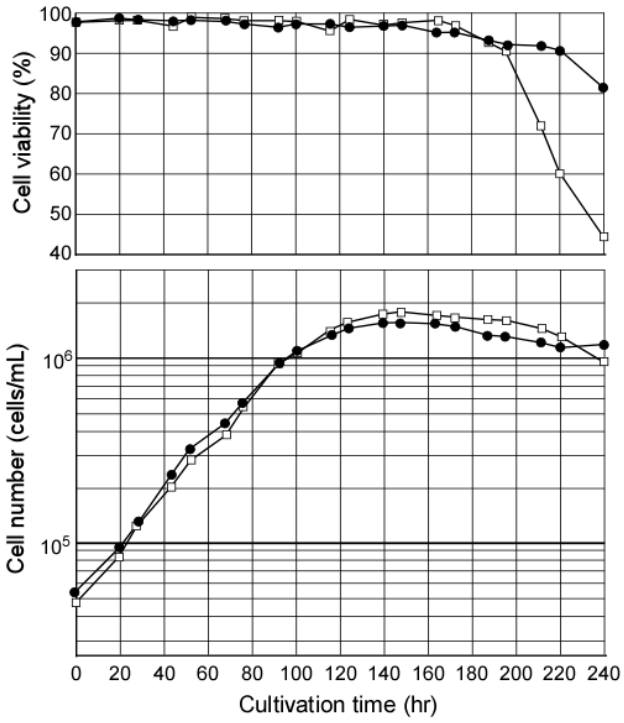

Fig. 6. Growth curves and cell viabilities of CHO 1-15 cells.

CHO 1-15 cells were cultivated in bioreactors with EE (open rectangle □) or RM (closed circle ●). (A) Cell viabilities. (B) Growth curves. Batch culture was started at 4 × 10^4 cells/mL. There was no significant difference in terms of cell growth in either bioreactor; however, cell viability in EE bioreactor suddenly decreased at 200 h.

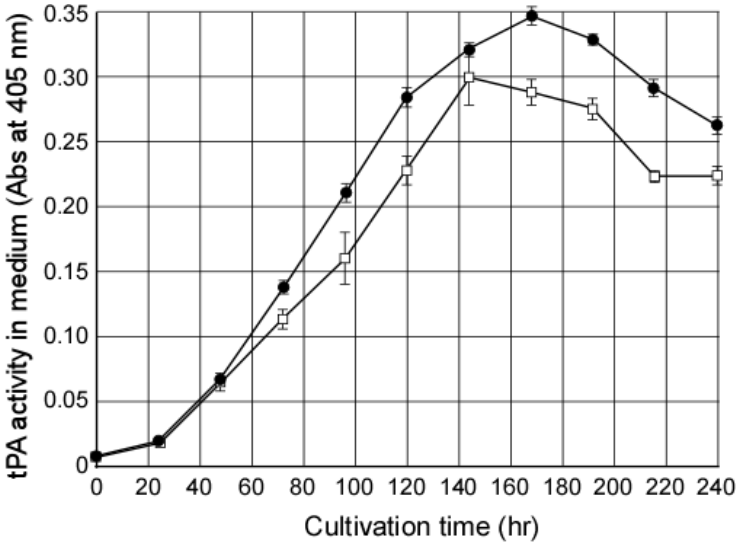

Fig. 7. tPA activity produced by CHO 1-15 cells.

Production of tPA by CHO 1-15 cells began, along with cell growth, in the bioreactor with EE (open rectangle □) and RM (closed circle ●).

Table 1. Summary of CFD analysis in bioreactors with EE or RM.

	Shear stress (Pa)		Shear rate (1/s)		Turbulent energy (m^2/s^2)		Energy dissipation (m^2/s^3)		Kolmogorov scale (μm)	
	Average	Max.	Average	Max.	Average	Max.	Average	Max.	Average	Max.
EE	1.20	20.9	18.6	2423	0.0038	0.032	0.022	4.4	105	21.9
RM	1.07	20.2	10.3	1359	0.0037	0.030	0.016	3.9	105	22.5