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Modified harvest system for enhancing Factor VIII yield in alternating tangential flow perfusion culture

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This study describes the development and experimental verification of a modified harvest system to enhance Factor VIII (FVIII) yield in an alternating tangential flow (ATF) perfusion culture. The main innovation of the modified harvest system is the use of check and pinch valves, eliminating the need of a peristaltic pump for harvest. The system was applied to perfusion cultures of Chinese hamster ovary cells, which co-express both recombinant human FVIII (rhFVIII) and von Willebrand factor (vWF). The modified harvest system showed comparable cell growth with the conventional harvest system using a peristaltic pump. The perfusion rate was successfully controlled using the system. In addition, the modified harvest system achieved an approximately 13.6-fold increase in the final concentration yield of FVIII activity and a 1.47-fold increase in the production yield of FVIII activity compared with a peristaltic pump. Enhancement of the yield of FVIII activity resulted from the reduction of FVIII antigen (FVIII:Ag) retention. As a result of transmembrane pressure (TMP) measurement, the reduction of the retained FVIII:Ag was due to the increased TMP, which was caused by the characteristic function of a check valve, compared with a peristaltic harvest system. The modified harvest system developed in this study could be useful to enhance the production yield of other recombinant proteins in ATF perfusion culture.

[**Key words:** Modified harvest system; Alternating tangential flow perfusion culture; FVIII yield; Pinch valve; Check valve; Transmembrane pressure; Filter fouling]

The perfusion mammalian cell culture process has been typically used as a manufacturing process of unstable recombinant proteins, such as recombinant clotting factors, enzymes used in enzyme replacement therapy and other proteins (1,2).

A number of cell retention devices are used to separate cells from the culture supernatants in perfusion culture. Most cell retention devices are based on filtration, gravitational sedimentation, centrifugation (centrifuges, hydrocyclones) and ultrasonic separation. There are many cell retention devices based on filtration process, such as cross-flow filter, vortex-flow filter, spin-filter and hollow fiber filter (3). Among the various cell retention devices, perfusion cultures with an external cell retention device are mainly used in the industrial production of the licensed rhFVIII preparations (4).

Presently, an alternating tangential flow (ATF) system was utilized as a cell retention device. The ATF system from Refine Technology is the newest perfusion device using hollow fiber filter for cell separation from the spent medium (5,6). The main features of the ATF system are self-cleaning and back-flushing action by a diaphragm pump. The repeated and rapid flow between hollow fiber inlets and bioreactor by a diaphragm pump inhibits the blockage of hollow fiber inlets. Also, back-flushing flow created by changing transmembrane pressure protects the hollow fiber filter from biofilm formation on its surface. These two main actions allow high cell density culture and extended perfusion time in ATF perfusion culture (7–9).

In filtration-based perfusion, such as ATF perfusion, the retention of the target protein inside the bioreactor due to a filter fouling is an inherent problem. Filter fouling occurs mainly because of the deposition of cell debris and nucleic acids in the perfusion culture of mammalian cells (10). There have been many attempts to decrease the retention of the target protein by the reduction of filter fouling in filtration-based perfusion cultures. A review of many strategies for improving filtration efficiency is given elsewhere (10).

Although the ATF system is designed to minimize filter fouling by diaphragm pump action, fouling problems were also observed in ATF perfusion culture. The concentration difference of high molecular weight antibody across the membrane surface was observed in a hollow fiber bioreactor, although a back-flush was applied, as in the ATF system. The retention rate was up to 20% (11). Others reported that up to approximately 60% of the produced antibody was retained by hollow fiber filter in ATF perfusion culture (12). However, the authors did not suggest any solution to overcome the retention of target protein due to fouling. Recently, we also observed that the concentration yield of FVIII activity was

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significantly low due to the retention of FVIII:Ag in ATF perfusion culture. The average concentration yield of FVIII activity was 8.2% (13).

In this work, we developed a modified harvest system for ATF perfusion culture. The system consists of a check valve, pinch valve and timer. We compared the performance of the modified harvest system with a peristaltic pump and examined the relationship between the enhanced FVIII activity concentration yield and TMP across the hollow fiber membrane.

MATERIALS AND METHODS

Design of a modified harvest system Peristaltic pumps are mainly and generally used for the addition of fresh medium and/or the withdrawal of spent medium, i.e., perfusion rate control in perfusion cultures. Peristaltic pumping has

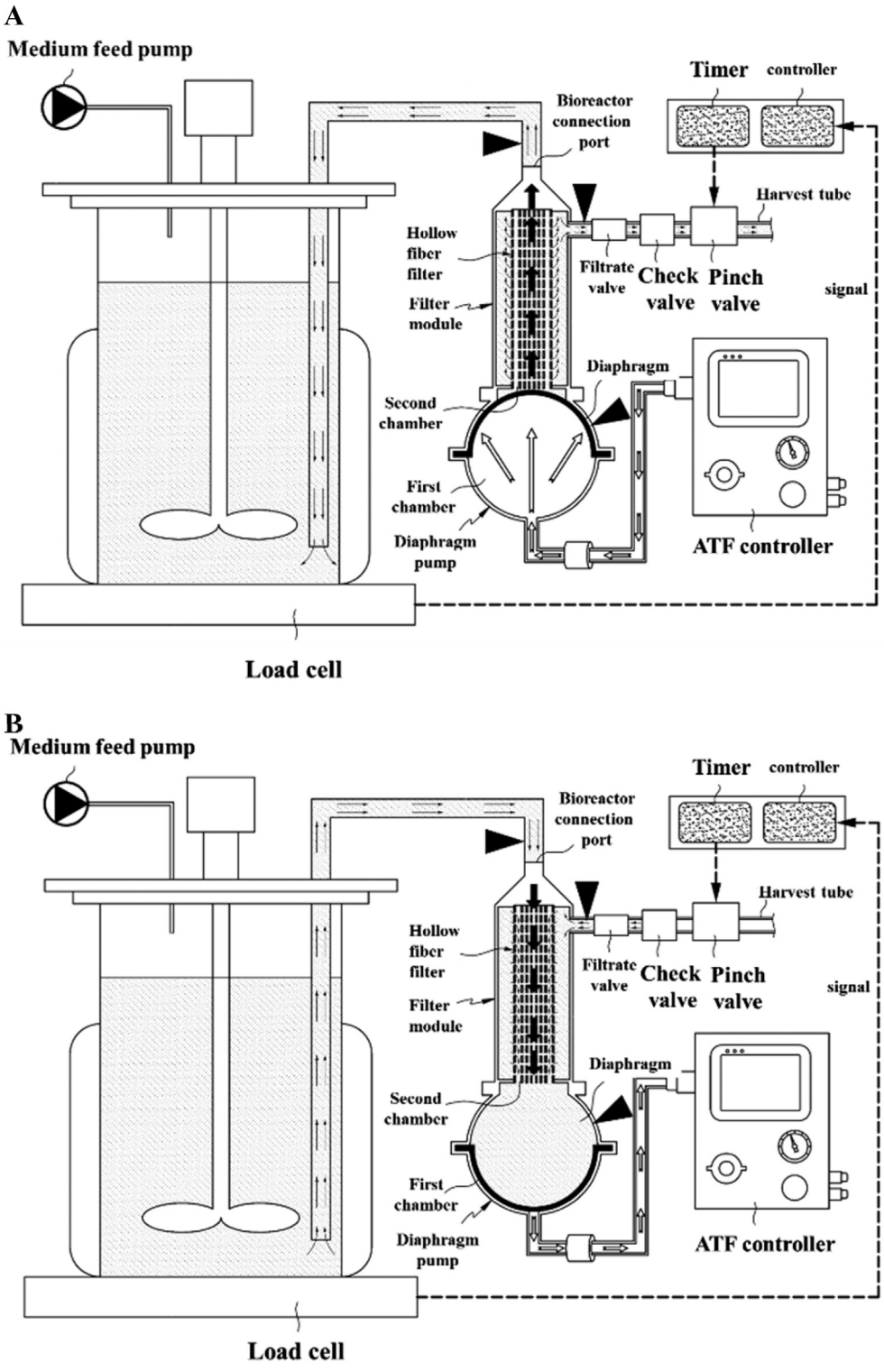

FIG. 1. Schematic diagram of ATF perfusion system with a novel harvest system. (A) Pressure cycle and (B) exhaust cycle of a diaphragm pump. The closed triangles indicate pressure transducers of KrosFlo Research Ili system.