

DESIGNING OF PERFUSION SYSTEM FOR MAMMALIAN CELL CULTURE

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**DESIGNING OF PERFUSION SYSTEM FOR
MAMMALIAN CELL CULTURE**

by

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Submitted
in fulfilment of the requirements of the degree of
Doctor of Philosophy

to the



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Dedicated to....

My parents Mr. Devendra Kamthan and Mrs. Asha Kamthan

&

My husband Vipul Ratan and children Navya and Nabh

CERTIFICATE

This is to certify that the thesis entitled “**DESIGNING OF PERFUSION SYSTEM FOR MAMMALIAN CELL CULTURE**”, being submitted by **Ms. SHWETA KAMTHAN** to the Indian Institute of Technology Delhi, for the award of degree of **Doctor of Philosophy**, is a record of bonafide research work carried out by her under my supervision and guidance in conformity with the rules and regulations of Indian Institute of Technology Delhi. The research reports and results presented in the thesis have not been submitted to any other university or institute for the award of any other degree or diploma.

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(Shweta Kamthan)

ABSTRACT

Mammalian cell culture is widely used for the production of various therapeutic proteins such as monoclonal antibody, tissue plasminogen activator, etc. For this, perfusion culture of mammalian cells using spinfilters has been primarily used. However, spinfilters currently being used are either highly prone to clog during perfusion operation and/or are disposable. This in turn lowers the therapeutic proteins productivity. To address this problem, a silk screen based reusable spinfilter module for clog-free extended perfusion operation of mammalian cells has been designed.

The spinfilter module has been designed using the biocompatible materials, stainless steel-316 and Teflon. Its cylindrical polymeric membrane supporting module is versatile in nature, as different polymeric membranes that meet specific process requirements according to the types of cells cultured can be mounted over it as its filter screen. The module is autoclavable, as all the materials used in its construction can withstand high temperature up to 121°C and reusable, as the same module can be used again after each perfusion experiment by autoclaving it after replacement of used filter polymeric membrane with the new one.

In this study, taking into account the biocompatibility of silk to mammalian cells, surface properties of different non-woven *Bombyx-mori* silks were studied and compared with other polymeric filter membranes. Among these, the Seri-DSS silk, which was found to be non-conducive to cells attachment due to its high hydrophobicity (water contact angle $108.2 \pm 1.1^\circ$), negative surface charge density ($-38 \pm 0.46 \text{mV}$) and suitable pore size range ($14.87 \pm 1.8 \mu\text{m}$) was selected and used as the filter screen of the designed spinfilter module. The application of silk spinfilter for clog-free extended perfusion culture of mammalian cells was demonstrated by comparative perfusion experiments with conventional stainless-steel spinfilter using both non-adherent HB8696 hybridoma cells and adherent HT1080 human kidney cells.

From the results, it was found that owing to its hydrophobic and negative filter screen, silk spinfilter was less prone to clog and foul with the cells, cell debris and media components compared to the positive charged hydrophilic stainless-steel and thus it provides extended clog-free perfusion operation of both non-adherent and adherent mammalian cells compared to the stainless-steel spinfilter.

For perfusion cultures of non-adherent HB8696 cells, silk spinfilter extended the cell growth phase by 56 h and monoclonal antibody production phase by 40 h, compared to the stainless steel spinfilter and provided 57.4 % enhancement in monoclonal antibody productivity. It provided higher maximum viable cell density of $2.5 \pm 0.1 \times 10^7$ cells/ml and monoclonal antibody productivity of $1.48 \text{ g L}^{-1} \text{ day}^{-1}$ compared to the maximum viable cell density of $1.3 \pm 0.1 \times 10^7$ cells/ml and monoclonal antibody productivity of $0.94 \text{ g L}^{-1} \text{ day}^{-1}$ achieved using the stainless steel filter.

Similarly, for perfusion cultures of adherent HT1080 cells, silk spinfilter extended the exponential phase by 48 h and production phase by 16 h compared to the stainless-steel spinfilter and provided 30.2% increase in urokinase productivity. It provided higher maximum viable cell density of $2.1 \pm 0.1 \times 10^7$ cells/ml and urokinase productivity of 792.4 PU/ml/day compared to the maximum viable cell density of $1.2 \pm 0.1 \times 10^7$ cells/ml and urokinase productivity of 608.5 PU/ml/day achieved using the stainless steel spinfilter.

To enhance the therapeutic protein productivity using silk spinfilter, its performance at different perfusion rates ($0.625 \text{ ml min}^{-1}$, 0.75 ml min^{-1} , $1.125 \text{ ml min}^{-1}$ and 1.5 ml min^{-1}) was studied using non-adherent HB8696 cells and the optimum perfusion rate for high monoclonal antibody productivity was determined. Among these rates, the perfusion rate of $1.125 \text{ ml min}^{-1}$ was found to be the optimum one, as at this rate the maximum viable cell density of $3.5 \pm 0.1 \times 10^7$ cells/ml, maximum monoclonal antibody concentration of $793 \pm 22.2 \text{ mg/L}$ and an improved monoclonal antibody productivity of $1.6 \text{ g L}^{-1} \text{ day}^{-1}$ was achieved.

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