DESIGNING OF PERFUSION SYSTEM FOR MAMMALIAN CELL CULTURE

SHWETA KAMTHAN

DEPARTMENT OF BIOCHEMICAL ENGINEERING AND BIOTECHNOLOGY
INDIAN INSTITUTE OF TECHNOLOGY DELHI
HAUZ KHAS, NEW DELHI-110016
OCTOBER 2014

DESIGNING OF PERFUSION SYSTEM FOR MAMMALIAN CELL CULTURE

by

SHWETA KAMTHAN

Department of Biochemical Engineering & Biotechnology

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

to the

INDIAN INSTITUTE OF TECHNOLOGY DELHI
OCTOBER 2014

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.

Dr. Pradip K Roychoudhury

Professor,

Department of Biochemical Engineering and Biotechnology,

Indian Institute of Technology Delhi

Hauz Khas, New Delhi - 110016.

DATE

NEW DELHI

ACKNOWLEDGEMENTS

I would like to thank my supervisor Prof. Pradip Kumar Roychoudhury, Department of Biochemical Engineering & Biotechnology, IIT Delhi for providing me his valuable guidance and support to complete my research work and thesis writing. I am indebted to Prof. James Gomes from Kusuma school of Biological Sciences, IIT Delhi for his innovative thinking that has given a marked direction to this research work and his support during the entire duration of my PhD. I would like to thank the SRC members of my research advisory committee: Prof T. R .Sreekrishnan form Department of Biochemical Engineering and Biotechnology, IIT Delhi, Prof. Aditya Mittal from Kusuma School of Biological Sciences, IIT Delhi and Prof. Ashok Mukhopadhyay from National Institute of Immunology, Delhi for their important suggestions and review.

I would like to thank Prof. Biswajit Kundu from Kusuma School of Biological Sciences, IIT Delhi for his significant efforts for the continuity of this project. I thankfully acknowledge the financial support from IIT Delhi and DBT, New Delhi. I am sincerely thankful to the technical in-charge Rana sir and my lab assistants Vinod and Sunil Kumar for their support and help in everyday practical work and running the bioreactor. I would like to thank all faculty members, labmates, seniors, and official staff for their help and support

I would like to recognize the unconditional support and inspiration of my parents and my husband, Vipul and love and patience of my children, Navya and Nabh without whom this would have been impossible. I would not have completed my PhD without their unending patience, love and support. I am deeply indebted to my family (grandmother, parent-in-laws and brothers Prashant and Praveen) for their belief, encouragement, understanding and above all blessings that could only make this possible. Last but not the least I thank the almighty God who blessed me the opportunity for this work and ability for its completion.

(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±1.1°), negative surface charge density (-38 ±0.46mV) and suitable pore size range (14.87 ± 1.8µm) 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 $2.5\pm0.1\times10^{7}$cells/ml and monoclonal antibody productivity of 1.48g $L^{-1}day^{-1}$ compared to the maximum viable cell density of $1.3\pm0.1\times10^{7}$cells/ml and monoclonal antibody productivity of 0.94 g $L^{-1}day^{-1}$ 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 $2.1\pm0.1\times10^{7}$cells/ml and urokinase productivity of 792.4 PU/ml/day compared to the maximum viable cell density of $1.2\pm0.1\times10^{7}$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 (0.625ml min^{-1}, 0.75ml min^{-1}, 1.125ml min^{-1} and 1.5ml 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 ml min^{-1} was found to be the optimum one, as at this rate the maximum viable cell density of $3.5\pm0.1\times10^{7}$ cells/ml, maximum monoclonal antibody concentration of 793 ± 22.2 mg/L and an improved monoclonal antibody productivity of 1.6g $L^{-1}day^{-1}$ was achieved.

TABLE OF CONTENTS