CHAPTER 1

Copyright
IIT Kharagpur

INTRODUCTION

This thesis focuses on the development of a support matrix for animal cell culture overcoming the lack of microenvironment in large scale cultures. These types of approaches are expected to solve the central problem of low cell population density in a culture vessel. Since, product concentration in the culture is directly related to the population density of cells, it is important to increase the cell concentration in unit volume of culture vessel for commercial exploitation of cell culture based products. For future pharmaceutical industry large scale animal cell culture based process is an attractive option. Recombinant DNA expression systems other than animal cells for the newly developed pharmaceuticals are found to be less efficient, limiting their commercial prospects. In case of animal cell expression system it is more promising from the point of view of proper product formation and its acceptability (Katinger and Scheirer 1985).

Adaptation of a number of conventional products like viral vaccines (poliomyelitis and rabies), insulin, complement factors and antisera to culture base processes instead of direct extraction from animals are necessary from quality control and ethical point of views. Although, it still may be cheaper to produce such products in third world countries by conventional methods, yet, for global market these practices are unacceptable.

On the other hand cell culture based processes have the potential for scale-up as unit process, which is not possible in case of extraction from animals. Demand for pharmaceuticals such as recombinant proteins and vaccines through cell culture are increasing steadily (Katinger and Bleim

1983) and present requirements are not met due to slower development in reactor technology and down stream processing.

At present available technological options are low yielding, either due to small size of culture or low cell population density per unit volume of culture. Hence, there is a need for large scale, high cell density culture system with higher product output (Katinger and Scheirer 1985).

Theoretical possibility exists in nature to support the argument in favor of a conceptual system. In an animal organ average cell density can be as high as $2x10^9$ cells per cm^3 volume of tissue (assuming cell dimension 8 μm to 10 μm and no interstitial space between them). Cells are present in highly compact manner and their metabolic activities are supported by the network of arteries, veins and extracellular matrix (Spier and McCullough 1987).

A typical conventional large scale system (microcarrier technology) has cell population density in the order of 10^6 to $2x10^6$ cells/mL of culture (Runstadler and Cernek 1988). This is very low for a product to be isolated economically to meet the demand. Due to these reasons animal cell culture processes are not yet commercially competitive.

Animal cells have lower specific growth rate and product yield than microorganisms, therefore, for achieving appreciable product output it is necessary to increase cell number per unit volume of culture and also the culture volume (Merten 1987).

One of the recent options is to mimic extra cellular matrix (ECM) for the cell culture in a better microenvironment. The proposed matrix should resemble the extra cellular matrix both physically and functionally for the successful cell growth at high densities. The materials for such matrices can

be chosen for their resemblance with extracellular matrix components (Cahn 1990).

In the present study the development and demonstration of such a technique to achieve high cell density per unit volume have been presented. The work has been divided into two parts. **First part** deals with the development of a suitable support matrix for cell culture (porous microcarrier) to provide better microenvironment and protection against shear stress.

The basic approach used to achieve these objectives are to mimic the *in vivo* conditions of extra cellular matrix by using suitable biomaterials. If the properties of the materials chosen are similar to collagen and other biopolymers present in the ECM, it may achieve the similar microenvironment in porous microcarriers, and perhaps the cell population density equivalent to *in vivo* tissues.

Second part of the thesis mainly deals with culture experiments. Utility of this porous matrix for the culture of various cell types has been established. Effect of various parameters on batch culture conditions for this new type of microcarrier was studied with one of the industrially useful cell lines. Finally, a comparison has been made with solid microcarrier to evaluate its relative advantages over conventional microcarrier process.

It will be appropriate to mention here that the presentation of entire work has not been restricted to the conventional way. Systematic presentation of the first part was difficult due to large number of trial and error type experiments. Results, discussion and conclusions have been merged at many places to keep the clarity of presentation.

In the second part again similar approach is followed. It was convenient to discuss results after description of a particular experiment

without giving separate headings, instead of pooling all the results and discussions of various experiments under a single heading.

Copyright IIT Kharagpur

AIMS AND OBJECTIVES

In order to achieve high population density of animal cells using a porous matrix, quantum of work has been divided in two parts under the chapter 3 & 4.

3. **Development of a porous matrix for cell growth.**
4. **Evaluation of the porous matrix for high population density culture of animal cells.**

A detailed outline of aims and objectives are given below with their section numbers under corresponding chapters.

DEVELOPMENT OF POROUS MATRIX

Major considerations for the development of a porous matrix are as follows.

3.1. Selection of biocompatible polymer.
3.2. Morphological aspect of porous matrix.
3.3. Strategies to make porous matrix.
3.4. Experiments with selected polymers.
3.5. Preparation of porous microcarrier.
3.6. Characterization of porous gelatin microcarrier matrix.
3.7 Conclusions

Copyright IIT Kharagpur

CULTURE EXPERIMENTS

4.1. Culture of cell lines on the porous matrix.

4.2. Study of batch culture conditions for porous microcarrier.

4.2.1. Effect of inoculum.

4.2.2. Effect of porous microcarrier concentration.

4.2.3. Effect of agitation during culture.

4.3. Comparison of cell culture on porous microcarrier with solid microcarrier.

4.3.1. Cell attachment kinetics

4.3.2. Lag phase of growth

4.3.3. Exponential phase of growth

4.3.4. Shear protection

4.3.5. Death Phase

4.5. Conclusions

5. FUTURE PROSPECTS

Copyright IIT Kharagpur