Nerve and Muscle Cells on Microcarriers in Culture

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Commercially available microcarriers (MCs), which are briefly described here, are usually used as a support for culturing a variety of cells both on laboratory and on an industrial scale. Selected MCs are used as a culture system for embryonic dissociated CNS cells and myoblasts. The tridimensional support provided by the MCs, enables neuronal and muscle cells to grow and differentiate into functional cell-MC units, which remain floating in the culture medium. The neuronal entities are characterized by intensive fiber growth followed by synaptogenesis and myelination. The muscular units develop striated myotubes, having the same orientation, which contract spontaneously. The MC technique is advantageous over the conventional monolayer procedure, allowing cells to grow to higher amounts at a better efficiency for longer periods. Functional units can be sampled without interfering with the ongoing culture. We consider the use of these cultures, as well as nerve muscle MC co-cultures as a tool for the study of neurotoxicology. The possibility of implantation of such functional culture units into injured adult nerve and muscle tissues is being considered.

Abbreviations

AChE Acetylcholinesterase
CNS Central nervous system
DIV Days in vitro
MC Microcarrier
MCs Microcarriers
SEM Scanning electron microscope or micrographs
TEM Transmission electron microscope or micrographs
1 Introduction

The development of microcarrier (MC) technology in tissue culture originated from the need for mass cultivation of mammalian cells in order to produce commercially biological cell products like viral vaccine, beta interferon etc. The concept of the microcarrier culturing system was first introduced by van Wezel in 1967. In this method cells were propagated on the surface of small solid particles suspended in the growth medium by slow agitation. The cells attached to the MC and grew gradually up to confluency on their surface.

Several cultivation systems were used for propagating cells on MCs. On a laboratory scale, cells were propagated on MCs in stationary Petri dishes, shake flasks, roller bottles and spinner flasks. On an industrial scale, cells were cultivated in fermentors up to 1000 liters volume. A wide range of cells from invertebrate, fish, bird and mammalian origin, transformed and normal cells, cell lines and lately genetically modified cells, have been cultivated on a variety of MCs.

The main advantages of this technology over monolayer cultures are: Availability of high surface to volume ratio which can be varied by changing the MC concentration; cells grow in a homogeneous culture in which environmental conditions are monitored and controlled; representative sampling is easily performed during cultivation; cell propagation can be carried out in a single high productivity vessel, rather than using many small low volume units; and the system can be relatively easily scaled up. A detailed description of the microcarrier technology and its advantages has been presented by Reuveny. As mentioned previously, MCs are used mainly for mass cultivation of cells for production of viral vaccines and biologicals. However, recently, MCs are found to be an important tool in cell culture research, with various kinds of applications. Several laboratories have shown that cells can be sub-cultivated by transfer from MCs to the dish without application of proteolytic enzymes. Duda has shown that cells can be cryopreserved, while attached to MCs. Microcarrier cultures are processed for transmission (TEM) and scanning (SEM) electron microscopy.

Crespi and Thilly have used synchronized mitotic cell population to study cell cycle dependence of certain mutagenic chemicals. Cells on MC are used in membrane isolation and membrane research, and for studies of cell-cell and cell-substratum interactions. Kotler et al. have shown that non-transformed cells which grow in dishes as monolayers can grow on MCs in multilayers. They suggested that the ability of cells to grow in multilayers is determined not only by their state of transformation but also by the type of support they are growing on. Davis and Kerr and Davis et al. have co-cultivated vascular endothelial cells and smooth muscle cells, using MCs, in order to study interaction between these two types of cells. Ren used MCs for depleting macrophages from mouse spleen cell population.

The ability to propagate differentiating cells at high densities in a three dimensional mode on MCs rather than as bidimensional monolayers, provides a unique opportunity for studying cell differentiation, interaction and function. Differentiating bone cells, thyroid cells, pituitary cells, pancreatic beta-cells, endothelial cells, chick embryo muscle cells and mouse peritoneal macrophages, were propagated on cylindrical and beaded MCs. These cells were propagated in batch cultures.