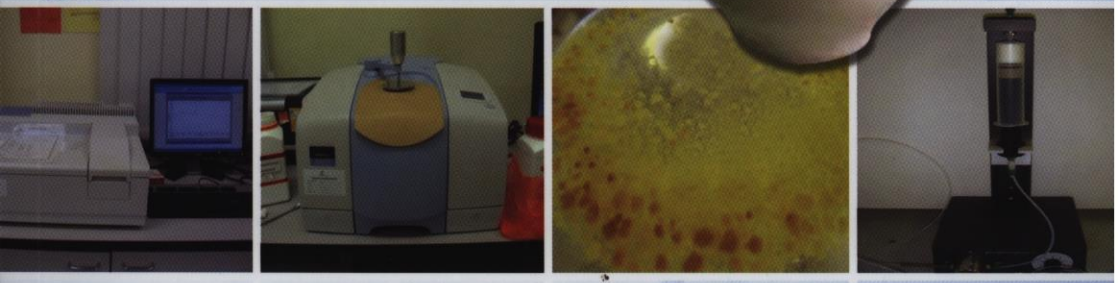
# EXPERIMENTAL METHODS in MODERN BIOTECHNOLOGY

Volume 2



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**IIUM Press**

Gombak • 2016

First Edition, 2016


IIUM Press is a member of the Majlis Penerbitan Ilmiah Malaysia – MAPIM
(Malaysian Scholarly Publishing Council)



Perpustakaan Negara Malaysia Cataloguing-in-Publication Data

Parveen Jamal
Experimental Methods in Modern Biotechnology.
Volume 2 / Prof. Dr. Parveen Jamal.
ISBN 978-967-418-384-4
1. Biotechnology. I. Title.
660.6

Published by:
IIUM Press
International Islamic University Malaysia
P.O. Box 10, 50728 Kuala Lumpur, Malaysia
Tel: +603-6196 5014; Fax: +603-6196 4862/6298

Printed in Malaysia by:
REKA CETAK SDN. BHD.
12 & 14, Jalan Jemuju Empat 16/13D
Seksyen 16
40200 Shah Alam, Selangor, Malaysia

# Contents

# Chapter 1

# Simple Cell Attachment and Viability Assay for Screening Anticancer Properties of Natural Products

Yumi Zuhanis Has-Yun Hashim
Chris Gill

## Introduction

Attachment to an extracellular matrix (ECM) and basement membrane is one of the initial processes in tumour invasion and metastasis. Cell attachment or adhesion is mediated by specific cell surface receptors for molecules in the ECM. Trypsin (or trypsin-EDTA or equivalent) is used to dissociate the cells from the cell-cell and cell-matrix interaction. Following tissue disaggregation, adherent cells will need to attach and spread out before they can start to proliferate. In normal T-flasks, cells would have to produce own ECM and then bind to it. In this particular attachment assay, cells are incubated for 24 hours, trypsinised and then counted using trypan blue dye exclusion method. If there were anti-attachment effect of compounds tested, number of viable cells counted would decrease (as compared to control). This simple procedure provides rough information about the adhesion system and the overall effect of anti-attachment, but does not give insights of complex interactions.

Once the cells adhered to the substrate, they will start to proliferate. For cancer cells, proliferation rates are very high. In order to prevent or stop further progression of cancer, cytotoxic agents are given to kill the cancer cells. Cell viability assay can be used to study potential cytotoxic effects of certain plant extracts or compounds of interest. When performed using cancer cell lines, the cytotoxic effects can be carefully translated into anticancer effects. Since this method is based

on the cell count at the end of the incubation period, the effects seen (in the presence of compounds of interest) could actually be a combination of inability of cells to attach to substrate in the first place, detachment of cells, cytotoxicity and inhibition of cell growth. By performing both cell attachment and cell viability on the compounds of interest, one can determine whether the effects are at the attachment level or killing the already adherent cells (cytotoxicity effects). However, additional assays are required to investigate the possible effects of the compounds in inhibiting cell growth which is not in the scope of this chapter.

## Advantages and Disadvantages of Cell Attachment and Viability Assays

The advantages of both methods include its simplicity and straight forward analysis based on cell count. The assays also only require common reagents, consumables and apparatus in a mammalian cell culture lab. Furthermore, the large surface area (in the T-flask) allow considerable number of cells to be tested upon compounds of interest, thus, avoiding false positive results that may occur in conditions where cells are too few. The use of considerable large volume of media also prevents fold over effects that would normally occur in miniaturised *in vitro* assays.

However, the disadvantages of the assays include high potential of human error in cell counting. Another drawback of the assays is high cost due to the use of large volume of media and cells as well as T-flasks (particularly when the experiment involves various concentrations of compounds to be tested). The assays are also not suitable for suspension (non-anchorage) type of cells.

## Materials and Methods

### Materials, Apparatus and Equipment

Materials needed are plant extract or compound of interest at known concentration, 70-80% confluent mammalian cells of interest (cancer