An easy-to-handle semi-automated method for media development using a colorimetric viability assay and fractional factorial designs

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Abstract

A rapid and effective semi-automated screening method has been developed for the development of growth media for mammalian cell culture. The method has proven to be a powerful tool for preliminary evaluation and comparison of new media formulations, but has some inherent disadvantages, which are important to recognize in order to interpret the results.

Abbreviations: AUC: Area under curve; DMEM: Dulbecco’s modified eagle’s medium; DMF: N,N-Dimethylformamide; ELISA: Enzyme linked immunosorbent assay; FCS: Fetal calf serum; HEPES: Hydroxy-ethyl-piperazine-ethane-sulphonic acid; MTT: 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyl-tetrazolium bromide; PEG: Polyethylene glycol; SDS: Sodium dodecyl sulphate.

Introduction

The economical production of pharmaceuticals in mammalian cell culture largely depends on the development of new culture media, which are optimal for both growth and production. Furthermore there is a need for cheaper and more well-defined media. Since media for mammalian cells are very complex mixtures of amino acids, carbohydrates, salts, vitamins, hormones etc., optimization of such media requires tests of a lot of different media formulations and can be a very labour- and time consuming work. Therefore an efficient practical approach is needed in order to minimize the work.

We here propose an easy-to-handle microscale method for media development, which can be implemented in any laboratory that has an ELISA-reader and a computer with a spreadsheet at its disposal.

The method described consists of two major components: a) a viability assay that measures the metabolic activity based on the reduction of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-tetrazolium bromide (MTT) by active mitochondria; and b) a statistical design that allows a relatively large number of parameters (factors) and their interactions to be analyzed and compared in relatively few experiments.

The MTT method is based on the reduction of a yellow water soluble tetrazolium salt MTT by active mitochondria. The reduction product is a blue, water insoluble formazan that after solubilization can be quantified spectrophotometrically.
A method which uses MTT for measurement of the viability of mammalian cells was proposed by Mosmann (1983). The method has mostly been used for screening of growth inhibitory compounds such as anticancer drugs, but has also been used for measuring the metabolic activity of cells (Gerlier and Thomasset, 1986) and for optimization of new cell culture media (Al-Rubeai and Spier, 1989; Mignot et al., 1989). We wanted to further investigate the method and to combine it with a systematic use of factorial designs for media development. The method seemed well suited for our purpose, because it can be semi-automated by using microtiter plates, multichannel pipettes and ‘ELISA-readers’, and thus makes it possible to perform a large number of experiments simultaneously.

We have used the MTT method combined with factorial designs in a systematic way by successively choosing the best medium/media from one assay as the basis for the next assay, and have in this way developed new serum-free media for several cell lines including mouse-mouse hybridomas, CHO- and BHK cells.

Materials and methods

Cell lines

In this study we used two mouse-mouse hybridomas, NUC 1–2 and NUC 1–4, both producing monoclonal IgG against nuclease from *Serratia marcescens* (Andresen et al., 1989). The cells used for the investigations of the MTT method were cultured in DMEM including 5% FCS supplemented with 0.9 g l\(^{-1}\) L-glutamine, 70 \(\mu\)M \(\beta\)-mercaptoethanol, 2.383 g l\(^{-1}\) HEPES, 0.122 g l\(^{-1}\) pyruvate and 3.7 g l\(^{-1}\) NaHCO\(_3\). Both cell lines were maintained in plastic tissue-flasks (Tec-Nunc, Denmark) and diluted every 3 to 4 days with fresh culture medium. The cells used for the media screening assay had been cultured in a serum-free medium called B1 containing 0.8% w/v casein peptone (Orthana A/S, Denmark) for several months before the experiment. The serum-free B1 medium is based on the medium described above but supplemented with 2.0 g l\(^{-1}\) dextran (Mw 2,000,000), 2.0 g l\(^{-1}\) PEG (Mw 70,000) and 5.0 mg l\(^{-1}\) human transferrin (Sigma).

MTT Assay

**Chemicals.** MTT was obtained from Sigma chemicals. The MTT stock solution was prepared by dissolving the chemical in phosphate buffered saline (Biochrom) at a concentration of 5 mg ml\(^{-1}\). The solution was sterilized by filtration through a 0.22 μm filter (Sartorius Minisart NML) and stored at 4°C in the dark for no longer than 2 weeks. The solvent was prepared by dissolving SDS (Sigma) in a 1:1 mixture of water and DMF (Merck) at a concentration of 20% w/v. pH in the solvent was adjusted to approximately 2 by addition of 1 N HCl/80% acetic acid.

**Procedure.** The procedure used is a modification of the MTT method (Mosmann, 1983), described by Hansen *et al.* (1989). 25 μl of the MTT stock solution was added to each well of a 96 well microtiter plate containing 125 μl cell suspension/well. The plate was then incubated for 2 h at 37°C, and the formazan produced by the cells dissolved by adding 150 μl of the solvent and incubating the plate overnight. The following day the absorbances were measured in an ‘ELISA-reader’ (Anthos 2001 reader, Anthos Lab. Instruments) at 570 nm with a reference wavelength of 690 nm (in the following designated OD\(_{570-690}\)). The data were transcribed to a diskette and transferred to LOTUS 123 (ver. 2.2; Lotus dev. corp.) where the data analysis was performed.

Statistical set-up

Multifactorial systems with interactions can be analyzed using factorial designs where all combinations of the factors are tested. The class of factorial designs that are most easy to perform are two-level designs where each factor is tested at two pre-defined levels e.g., two concentrations. The number of experiments that are necessary in order to investigate \(k\) factors is thus \(2^k\). With