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Three-dimensional chitosan scaffold-based MCF-7 cell culture for the determination of the cytotoxicity of tamoxifen

Harpreet K Dhiman[a,b], Alok R Ray[a,b], Amulya K Panda[c,*]

[a] *Centre for Biomedical Engineering, Indian Institute of Technology, New Delhi 110016, India*
[b] *All India Institute of Medical Sciences, New Delhi 110029, India*
[c] *National Institute of Immunology, Aruna Asaf Ali Marg,New Delhi 110067, India*

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Abstract

Three-dimensional (3D) culture of cancer cell lines has long been advocated as a better model of the malignant phenotype that is most closely related to tumorigenicity in vivo. Moreover, new drug development requires simple in vitro models that resemble the in vivo situation more in order to select active drugs against solid tumours and to decrease the use of experimental animals. A biodegradable, biocompatible and non-toxic polymer chitosan was employed for 3D culture of MCF-7 cell lines. Cells grown on chitosan scaffold produce more lactate from glucose in comparison to that secreted by cells grown on tissue culture plate, thus indicating the suitability of chitosan scaffold as an in vitro model resembling cancer tissue growth in vivo. Cytotoxic effect of tamoxifen at different concentrations was evaluated for MCF-7 breast cancer cell lines grown on tissue culture plate as well as on 3D chitosan scaffold. At a tamoxifen concentration of 10^{-6} M, 50% reduction in cell growth was observed in tissue culture plate-grown cells where 15% reduction in cell growth was observed when cells were grown in chitosan scaffold. Higher tamoxifen concentrations were required to achieve comparable cytostatic action in 3D culture, supporting the fact that 3D culture is a better model for the cytotoxic evaluation of anticancer drugs in vitro. Carbohydrate metabolism of MCF-7 cells in terms of glucose utilization and lactate production in 3D and monolayer culture were unaffected by tamoxifen treatment. Cathepsin D activity, an autocrine growth factor in breast cancer cells was monitored in all experiments. In 3D culture, addition of tamoxifen promoted cathepsin D secretion but inhibited its uptake by cells. Growth of cells in 3D chitosan scaffold indicated that action of tamoxifen on estrogen positive cancer cells is also mediated through inhibition of cathepsin D uptake from the culture medium.

Keywords: Chitosan scaffold; MCF-7 cell lines; Three dimensional culture; Tamoxifen; Cathepsin D

1. Introduction

Three-dimensional (3D) culture of animal cells on polymeric scaffold-based extracellular matrix is a better alternative model for understanding cell metabolism [1]. In vitro animal cell growth in 3D promotes normal epithelial polarity and differentiation [2], cells move and divide more quickly and have a characteristically asymmetric shape compared with that of cells in living tissue [3]. 3D culture of cancer cells allows to explore many basic questions related to cancer biology, as receptors for growth factors which play an important role in tumour development are expressed in different ways in comparison to the standard tissue culture plates [4,5]. For breast cancer, 3D culture provides a model system for understanding the regulation of cancer cell proliferation and for evaluation of different anticancer drugs [6,7]. The factors that control proliferation of breast cancer cell lines are complex and not yet well defined [8] and there are reports that breast cancer cells shows partial differentiation when grown in 3D culture [9]. Even though extensive work has been reported using 3D culture for understanding tissue architecture [6,10], very little has been published on the use of 3D culture as an in vitro model for the cytotoxic evaluation of anticancer drugs. There is a substantial amount of evidence that cells growing in 3D culture are more resistant to cytotoxic agents than cells in monolayer or

*Corresponding author. Tel.: +91-11-26703509; fax: +91-11-26162125.
E-mail address: amulya@nii.res.in (A.K. Panda).

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dispersed culture. Many studies have demonstrated an elevated level of drug resistance of spheroids culture compared with cells in monolayers [11]. Initially investigators attributed drug resistance of spheroids to poor diffusion of the drugs to interior cells but now it has been proved that only 3D culture accounts for drug resistance rather than mere inaccessibility to nutrients [12,13].

Factors which control the proliferation of breast cancer cell lines are not yet well defined as the growth is under the complex control of both estrogen and autocrine and paracrine growth factors [14]. Regulation of cellular proliferation by estrogen and growth factors [14,15], as well as growth inhibition by non-steroidal antiestrogen [16,17] is mediated by estrogen receptor. Tamoxifen is the most widely used drug in breast cancer therapy acts through these receptors [18–20]. The presence of estrogen receptors in most breast cancer cells thus makes them susceptible to growth inhibition by tamoxifen [21]. It is generally believed that tamoxifen has antiestrogenic activity and competes with estrogen receptors to inhibit cell growth [19]. Extensive studies have been undertaken using estrogen-receptor-positive MCF-7 cell lines to understand the molecular mechanism of tamoxifen action. With experiments carried out in monolayer culture in static T-flasks, where growth is limited not only due to space but also due to the build-up of local concentrations of inhibitory by-products of the cellular metabolism, a true picture of cellular proliferation and the mechanism of tamoxifen action thereof is not obtained. Apart from this, the two-dimensional (2D) culture does not provide information about the kinetics of the many growth factors of secretion and uptake, which are important for survival of cancer cells. Cathepsin D is an aspartyl protease abnormally secreted by breast cancer cells [22]. In breast cancer cell lines, only pro-cathepsin D is secreted [23] and shows pepstatin-inhibitable enzymatic activities at an acidic pH [24]. Cathepsin D activity has been reported to be associated with increased disease occurrence, metastasis and increased mortality, and may have a prognostic value to patients with breast cancer [25,26]. It has been demonstrated that the secreted pro-cathepsin D is an autocrine mitogen [23] and it's over expression in vitro increases the cell proliferation [27]. Recent reports reveal that cathepsin D cleaves chemokines and other imflamatory proteins in breast cancer cells and it down regulation by antisense gene transfer inhibits tumour growth [28,29].

Recently we have reported the growth of MCF-7 cell lines on 3D porous chitosan scaffold [30]. Our hypothesis was that in vitro grown cells in 3D scaffold will provide better information on metabolic activities of breast cancer cells than the 2D culture. In this we report, we use 3D chitosan scaffold-based MCF-7 cell culture for the evaluation of cytotoxic effect of tamoxifen. Growth profiles of MCF-7 cell line, glucose uptake rate and lactate production rate during the growth on 3D scaffold was determined to provide information on primary metabolic activities. Modulation of cathepsin D enzymatic activity of MCF-7 cells by tamoxifen in 3D culture was also examined and compared with that of cell growth in the tissue culture plates.

2. Materials and methods

2.1. Materials

Chitin, from prawn shells, was obtained from the Central Institute of Fisheries Technology, Cochin, India. RPMI medium, and fetal calf serum was from Gibco BRL, USA. Trypsin EDTA, Trypan blue, Crystal violet, haemoglobin, glucose and lactate kit were purchased from Sigma Chemicals (St. Loius, USA). Cathepsin D and Pepstatin A were also obtained from Sigma chemicals. Tamoxifen powder was a gift from Dabur India Limited, New Delhi. All other chemicals were of an analytical grade.

2.2. Preparation and selection of chitosan for 3D matrix

Chitosan was prepared from chitin by the deacetylation process using 50% (W/W) sodium hydroxide solution at 110°C for 5 h [30]. After this treatment, flakes separated from the alkali layer were extensively washed with MQ water to remove the traces of alkali. The resulting flakes were dried in a vacuum oven at 50°C for 72 h. Chitosan flakes were dissolved in 1% aqueous acetic acid solution and filtered through a sintered glass filter. Chitosan was precipitated from the resulting solution with 10% aqueous sodium hydroxide solution. The precipitate was washed several times with MQ water to remove the traces of alkali. The chitosan flakes were purified using the method described by Muzzarelli et al. [31] by Soxhlet extraction with methanol, MQ water, petroleum ether and acetone in that order, each for 24 h. Finally, the chitosan powder was dried in vacuum oven at 50°C for 20 h and stored in a desiccator. Chitosan matrix having an 80.9% degree of deacetylation was used for preparation of scaffold for 3D culture of MCF-7 cell lines; the detailed characterization has been described elsewhere [30]. Briefly, chitosan scaffold was prepared by precipitation of 1% chitosan solution in 0.1 M NaOH. The scaffold was washed extensively with MQ water, and then transferred to a freezing bath and lyophilized [32]. The dried chitosan scaffold was sterilized by autoclaving in PBS for 15 min at 121°C and used for the MCF-7 cell culture.