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Microarray platform affords improved product analysis in mammalian cell growth studies

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Abstract

High throughput (HT) platforms serve as cost-efficient and rapid screening method for evaluating the effect of cell culture conditions and screening of chemicals. The aim of the current study was to develop a high-throughput cell-based microarray platform to assess the effect of culture conditions on Chinese hamster ovary (CHO) cells. Specifically, growth, transgene expression and metabolism of a GS/MSX CHO cell line, which produces a therapeutic monoclonal antibody, was examined using microarray system in conjunction with conventional shake flask platform in a non-proprietary medium. The microarray system consists of 60 nl spots of cells encapsulated in alginate and separated in groups via an 8-well chamber system attached to the chip. Results show the non-proprietary medium developed allows cell growth, production and normal glycosylation of recombinant antibody and metabolism of the recombinant CHO cells in both the microarray and shake flask platforms. In addition, 10.3 mM glutamate addition to the defined base media results in lactate metabolism shift in the recombinant GS/MSX CHO cells in the shake flask platform.

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Conflict of interest statement

The remaining authors have no individual consulting agreements with Janssen and have performed this research under a grant from Janssen.

Ultimately, the results demonstrate that the high-throughput microarray platform has the potential to be utilized for evaluating the impact of media additives on cellular processes, such as, cell growth, metabolism and productivity.

Keywords

cell-based microarray; high through-put; CHO cells; media optimization; 3D cell culture

Introduction

Chinese hamster ovary cells (CHO) cells have been widely employed for industrial production of biopharmaceutical products such as monoclonal antibodies, hormones, cytokines, and blood-products [1, 2]. The critical goal of industrial production of biotherapeutics from CHO cells is production of copious amounts of properly post-translationally modified recombinant biotherapeutics from robust cells in a cost-effective manner. Strategies to increase productivity include cellular engineering of cell lines, sub-cloning to isolate high producing clones, gene-amplification systems (DHFR/MTX and GS/MSX systems), process development (batch, fed-batch, perfusion, etc.), and media optimization [1]. The large number of recombinant biotherapeutic candidates entering different stages of development has placed increasing pressure on the biopharmaceutical companies to accelerate cell-culture development to deliver this pipeline and contain development costs. Cell-culture development, including strain selection, process optimization (medium formulation), and scale up, has traditionally been a labor-intensive process that is limited by the number of conditions that can be tested.

In recent years, there have been significant efforts to develop high-throughput (HT), miniaturized cell-culture systems that can improve the efficiency of the development process by allowing HT parallel operation, while reducing the cost of reagents, thus permitting the exploration of a vast space of culture conditions. HT platforms serve as a cost-efficient and rapid screening method for evaluating effect of cell culture conditions and screening of chemicals, including novel drugs in cell culture systems [3-13]. In addition, commercial systems such as deep well, and AMBR/TAP systems have been utilized for evaluating cell-culture systems. However, to date, there is no universal platform for optimizing cell-culture development. A three-dimensional (3D) cell-culture chip (the microarray DataChip) was developed that can be utilized to perform rapid screening of the effects of culture medium on cell growth rate, maximum viable cell density, and protein expression levels. The microarray DataChip, which can contain up to 1,080 individual mammalian cell cultures on a microscope-size glass slide (chip), has been used to cultivate a wide range of human and animal cell lines (liver, breast, pancreatic, colon, neural) and primary cells (hepatocytes, astrocytes, cardiomyocytes), as well as embryonic and adult stem cells [4, 10]. The high-throughput cell culture platform has the potential to be utilized for drug discovery (e.g. evaluating impact of novel drugs), human toxicology studies (e.g. evaluating potent anthropogenic compounds) and media optimization (e.g. evaluating impact of media components on cell growth and recombinant protein production).

Media development has been used to optimize CHO cell growth and/or the transgene expression in bioprocesses [14-17]. Media components such as carbon source, nitrogen sources, copper sulfate, manganese sulfate, and zinc sulfate and bioreactor temperature, pH, and shear stress can also impact cell growth, productivity and post-translational modifications of the transgenic protein produced [2, 17-23]. There are two strategies to increase productivity in cell lines with media optimization (1) increase specific productivity (i.e., amount of recombinant monoclonal antibody produced per cell of a bioreactor/day) and (2) increase cell yield (i.e., the total integrated viable cell density (IVCD) for the culture duration). The aim of this study is to develop a high-throughput microarray DataChip platform for examining the effect of media components on cellular processes, such as, growth, productivity, metabolism and its potential application towards on-chip metabolic pathway studies. In this work, first, (1) a defined non-proprietary medium was optimized for growth of a recombinant monoclonal antibody-producing GS/MSX CHO cell line, followed by (2) optimization of a 3D high-throughput microarray platform for studying the effects of different concentrations of media additives on cell growth, metabolism, and productivity, and (3) validation of the 3D high-throughput platform in conjunction with the conventional scale-up shake flask platform. The 3D high-throughput platform consists of DataChip microcultures separated in groups via an 8-well chamber system attached to the chip. Live-dead assays performed on the microarray spots, in conjunction with IgG competition assays and metabolite analysis, provide quantitative information on cell growth, protein production, and metabolism. In addition, cell-conditioned media was utilized to study the glycosylation pattern of the product using hydrophilic interaction chromatography (HILIC) – Fourier transform mass spectrometry (FTMS).

Materials and methods

Optimization of a non-proprietary medium and adaptation of CHO cells in the non-proprietary medium

A recombinant GS/MSX CHO cell line (CHO-A1, which produces a proprietary monoclonal antibody immunoglobulin type G (IgG), 150 kDa), was provided by J&J, Janssen Biotech and used as the model cell line. In addition, the host GS/MSX CHO cell line (CHO-H1) was also provided by J&J, Janssen Biotech. A non-proprietary media previously designed for the growth of DHFR/MTX CHO cell lines was used as the starting point for this study [19]. The non-proprietary medium was modified for the growth of the model recombinant CHO-A1 cell line and host CHO-H1 cell lines. The non-proprietary medium (Table 1) was prepared in sterile glass bottles. Following addition of all the supplements, the medium was stirred for 1 h, and the pH was subsequently adjusted to 7.0. The media was filter sterilized and stored at 4 °C. Prior to use, the medium was supplemented with insulin (Sigma-Aldrich) and 1% Penicillin-streptomycin (Sigma-Aldrich). The host cells (CHO-H1) required addition of 20 ml of Glutamax supplement (Invitrogen) per 500 ml of medium, while the transfected cell lines were supplemented with 25 μM of GS inhibitor methionine sulphoximine (MSX).

The proprietary CHO cells, CHO-H1 and CHO-A1, were directly adapted to grow in non-proprietary medium. The cell lines were seeded in 47 ml of medium at 3×10^5 cells/ml in 125 ml polycarbonate Erlenmeyer flasks (Corning, Corning, NY) and cultured on orbital

shakers agitated at 125 rpm in a humidified 37° C incubator and 5% CO_2. Growth rates were analyzed by manual cell counting using trypan blue to estimate cell viability and viable cell density, according to the standard operating protocol provided by J&J, Janssen Biotech. Briefly, cells were diluted with equal volume of Trypan Blue solution and cells were counted using a hemacytometer and quantified using the following equation:

$$\text{Viable cells}/mL = (\text{viable cell number/squares counted}) \times \text{dilution factor} \times 10^4$$

Preparation of 3D microarray cultures

The CHO cells were cultured in three-dimensional (3D) microarrays by embedding them in alginate, and printing the solution mixture in 60 nl spots onto hydrophobic glass slides. Briefly, a poly-L-lysine (PLL)–$BaCl_2$ mixture was prepared by mixing a sterile 0.1 M $BaCl_2$ solution in deionized water with a 0.01% (w/v) sterile PLL solution (Sigma) in a 1:2 volume ratio. The PLL-Ba^{2+} mixture was spotted onto poly(styrene-co-maleic anhydride) (PSMA) coated glass slides using a MicrosSys 5100-4SQ microcontact microarray spotter (DigiLab), making a 6 × 8 × 8 patterned array of 60 nl spots on the chip and allowing them to dry. A CHO cell suspension in media was then mixed with a low viscosity alginic acid (Sigma) solution in deionized sterile water such that the final alginate concentration was 1% (w/v), and the concentration of cells in suspension was 3.3×10^6 cell/ml. Subsequently, the cell-alginate mixture was printed (60 nl/spot, 200 cells/spot) on top of the dried PLL-Ba^{2+} spots, which allowed the nearly instantaneous gelation of the alginate matrix. During the printing process, the humidity in the microarray chamber was maintained above 90% to prevent water evaporation of the spots. Following printing, the slide was fitted with a commercially available 8-well polystyrene medium chamber coated with a biocompatible adhesive (Lab-Tek II, Nunc) that allowed firm attachment of the chip to the chamber, and physically separated groups of spots within the slide. Each of the chambers, containing 48 cell-culture spots, was filled with 250 μl of non-proprietary medium. After media addition, the chambers were covered with gas-permeable sealing membranes (Diversified Biotech), and the chips placed in segmented dishes (Thermo Scientific Nunc) with the surrounding wells filled with sterile deionized water to prevent media evaporation. The cells were then cultured at 37 °C in a 5% CO_2 humidified incubator. Terminal harvest samples taken at different culture time points were generated by carefully removing the media from one of the replicate chips, weighing the resulting mass of media to assess the percentage of evaporation, and freezing the samples at -20 °C. Following media recovery, chambers could be easily removed with a slide separator.

Cell viability assay

Cellular viability and growth on the 3D microarrays was assessed via a live/dead viability/cytotoxicity kit for mammalian cells (Invitrogen). After complete media recovery, the slides were separated from the chamber, and subsequently rinsed twice with a wash solution (140 mM NaCl (Sigma-Aldrich) and 20 mM $CaCl_2$ (Sigma) at pH 7). The slides were then immersed in a 0.5 μM calcein AM/ethidium homodimer 1 solution to detect viable and dead cells via green and red fluorescence, respectively. After 45 min of incubation at room temperature, the dye was removed and the chips rinsed thoroughly with the wash solution

and then dried. The slides were finally scanned with a GenePix 4200A scanner (Molecular Devices) using 488 nm excitation, with a standard blue filter and a 645AF75/594 filter for detection of the green and red dye, respectively. The fluorescence intensity from the resulting image was quantified with the GenePix Pro 6.0 software (Molecular Devices), and the cell number in each spot estimated based on a 6-point “viable-cell” standard curve prepared on the same day of printing. Determination of viability required the fabrication of a separate calibration curve for dead cells. This was achieved by permeabilizing the cells in calibrator samples with 0.15% saponin for 30 min, followed by live-dead staining. The ratio of the ethidium homodimer 1 fluorescence signal from the viable-cell standards to the dead-cell standards (total number of cells) yields the viability of the calibrators.

Evaluation of media additives in microarray DataChip and shake flask platforms

Media additives, pyruvate (Invitrogen), MEM non-essential amino acids (Invitrogen), MEM vitamins (Invitrogen), Mn^{2+} (Sigma-Aldrich) and glutamate (Sigma-Aldrich) were added at appropriate concentrations and pH was adjusted to pH 7.0. The medium was sterilized by filtration with a 0.22 μm syringe filter and stored at 4° C. On the day of the experiment, media were set at room temperature and were supplemented with 2% HT supplement, 1% Penicillin-streptomycin and insulin. The media were utilized in DataChip and/or shake flask platforms.

For high-throughput evaluation of the effects of media additives using the DataChip, recombinant CHO-A1 cells were seeded onto the DataChip in the various media. The cells were cultured in a humidified 37° C incubator at 5% CO_2 for eight days. Throughout the growth period, samples were analyzed by live-dead viability assay to determine the growth rates. When the samples were sacrificed for live-dead assays, cell-conditioned media was collected and stored at -20° C for further analysis.

For validating the DataChip results, selected media additives were evaluated in parallel in a shake-flask platform. For shake-flask studies, recombinant CHO-A1 cells were seeded at 2×10^5 cells/ml in a volume of 47 ml of media in polycarbonate Erlenmeyer flasks (Corning) and cultured on orbital shakers agitated at 125 rpm in a humidified 37° C incubator at 5% CO_2. Cells were cultured until the cell viability dropped to 50%. Growth rates were analyzed by manual cell counting using trypan blue to estimate cell viability. Additionally, cell-conditioned media was collected periodically at different time-points and stored at -20° C for evaluating recombinant antibody production, and metabolite profiling.

Meso Scale Discovery (MSD) competition assay for IgG Titer

The amount of recombinant monoclonal antibody (IgG1) production was determined using a competition assay on MSD's low-capacity Protein A coated plates, in the presence of a SULFO-TAG™ –labeled IgG tracer.

SULFO-TAG™ –labeled IgG tracer was prepared by tagging positive control IgG with MSD SULFO-TAG NHS-Ester (Meso Scale Diagnostics #R91AN) according to the manufacturer's directions. Briefly, a 2-mg/ml solution of the IgG was prepared in preservative-free PBS, pH 7.9. The IgG solution was incubated with 32 nmol of MSD SULFO-TAG NHS-Ester (50 μl) at 23° C for 2 h in dark. This labeling reaction was purified

with a pre-calibrated 40K MWCO ZEBA spin desalting columns (Thermo Scientific #87766), according to the manufacturer's instruction. The conjugation ratio was calculated using the following equation:

$$\text{Conjugation ratio} = \frac{A_{455} \times MW_{IgG}}{\varepsilon_{sulfo-tag} \times C_{IgG}}$$

In this equation, A_{455} is the absorbance of the SULFO-TAG IgG conjugate at 455 nm, MW_{IgG} is the molecular weight of the protein, $\varepsilon_{sulfo\text{-}tag}$ is the extinction coefficient of the SULFO-TAG at 455 nm (15,400 $M^{-1}cm^{-1}$), and C_{IgG} is the concentration of the protein conjugate determined using the BCA assay. The antibody was aliquoted and stored frozen at -20° C.

SULFO-TAG™ –labeled IgG tracer (stored at -20° C) was used for determining IgG amount in the cell-conditioned medium. The IgG is a secreted antibody and collected in the cell-conditioned medium. Cell-conditioned medium (1 ml) was collected on specified days and corresponding viable cell density was noted. The cell-conditioned media was collected by centrifugation of the cells and cell-conditioned media at 200xg for 10 min and stored at -20° C. The cell pellet was discarded. The MSD IgG competition assay was performed on the cell-conditioned media according to the manufacturer's protocol (MSD® 96-Well competition assay kit for IgG, Meso Scale Diagnostics). Briefly, the low-capacity protein A-coated MSD plate was blocked with 100 µl of diluent 100 in each well, at R.T. for 1h, followed by removal of the diluent 100 by washing three times with 50 µl of PBS/0.05% Tween-20. Mixtures of equal volumes of SULFO-TAG™ –labeled IgG tracer and dilutions of control IgG tracer (ranging from 0 – 100 µg/ml) were used to generate a standard curve. The mixtures of cell-conditioned medium and control IgGs (50 µl per sample in each MSD well) were loaded on to the washed low-capacity protein A-coated MSD plate and incubated for 2 h with vigorous shaking (500 rpm) at room temperature. The MSD plates were washed three times with 50 µl of PBS/0.05% Tween-20 and 150 µL of 1X Read Buffet T was added to each MSD well. The MSD plate was analyzed on the SECTOR® Imager (Meso Scale Diagnostics). The amount of IgG present in cell-conditioned medium was calculated based on the standard curve. The data was analyzed with MSD DAT software, which fits the standard curves using a four parameter logistic fit with $1/y^2$ weighting:

$$y = b_2 + \frac{(b_1 - b_2)}{1 + (x/b_3)^{b4}}$$

where x is the concentration, b_2 the estimated response at infinite concentration, b_1 the estimated response at zero concentration, b_3 is the mid-range concentration, and b_4 is the slope factor.

Glycan analysis

N-linked oligosaccharides of the antibody were released by treatment with PNGase F (Sigma-Aldrich, St. Louis, MO, USA) overnight at 37 ^{0}C following the protocol provided

by Sigma-Aldrich. After releasing the *N*-glycans, the antibody was precipitated by addition of ethanol followed by centrifugation. The supernatant was dried in a SpeedVac and stored at -80 °C for liquid chromatography-mass spectrometry (LC-MS) analysis.

The released *N*-glycans were analyzed on a hydrophilic interaction chromatography column (2.0 × 50 mm, Phenomenex Luna HILIC, Torrance, CA) coupled with electrospray ionization on an LTQ-Orbitrap XL FTMS (Thermo Fisher Scientific, San Jose, CA). Mobile phase A was 5 mM ammonium acetate prepared with HPLC-grade water. Mobile phase B was 5 mM ammonium acetate prepared in 98 % HPLC grade acetonitrile with 2 % of HPLC grade water. An Agilent 1200 HPLC binary pump was used to deliver the gradient from 10 to 80 % A over 8 min at a flow rate of 250 μL/min after injecting the samples. The optimized MS parameters included a spray voltage of 4.2 kV, a capillary voltage of −40 V, a tube lens voltage of −50 V, a capillary temperature of 275 °C, a sheath flow rate of 30 l/m, and an auxiliary gas flow rate of 6 l/m. External calibration of mass spectra routinely produced a mass accuracy of better than 3 ppm. All Fourier-transform mass spectra were acquired at a resolution 60,000 within 400–2,000 Da mass range.

Metabolite analyses

Metabolite analyses were performed using either a YSI 7100 MBS (YSI Inc., Yellow Springs, OH) or a BioProfile FLEX (NOVA Biomedical, Waltham MA).

Data analysis

The specific growth rates, μ, were obtained from the exponential growth phase of the culture. This growth data, along with the titer values obtained from the MSD competition assay, allowed calculating the rate of mAb production in the exponentially growing culture. Specific productivities were extracted from the slope of a plot of integral viable cell density (IVCD) versus mAb concentration, where the trapezoid method was used to estimate the time integral of viable cell number:

$$IVCD = \frac{(X_i + X_{i-1})\,(t_i - t_{i-1})}{2},$$

where t is time and X is the viable cell concentration. The cell and mAb concentrations above were corrected for the measured media evaporation.

Data were analyzed with Student T test to determine statistical differences between independent samples.

Results and discussion

Development of a non-proprietary medium for growth of recombinant CHO cells

The first critical step of the project was to establish growth of the model CHO cell lines in a defined non-proprietary medium. A non-proprietary media that had been developed for the growth of DHFR/MTX CHO cell lines was used as the starting point [19]. This non-proprietary medium was modified for the growth of the GS/MSX CHO cells (Table 1).

During direct adaptation of the cells to the non-proprietary medium, it was observed that addition of 2% hypoxanthine-thymidine supplement (Gibco) was necessary to allow adequate cell growth, viability, and production of mAb. Cell growth and recombinant antibody production in the proprietary and defined non-proprietary medium were evaluated (Supporting Information Figure S1). In addition, the glycoprotein secreted from the CHO-A1 cell line grown on non-proprietary medium was collected from 20 ml of media recovered from shake flask culture. Treatment of recovered IgG glycoprotein with PNGaseF and analysis of released *N*-glycans showed three biantennary structures each with a bisecting fucose and containing 0, 1, and 2 sialic acid residues (Supporting Information Figure S2). Thus, the defined non-proprietary medium developed allows for normal glycosylation of IgG glycoprotein.

Design and optimization of a microarray DataChip platform for CHO cell growth

HT platforms for evaluating impact of media components on cellular processes, including recombinant protein production, are of critical importance. Based on the previous DataChip microculture platform [10], a 3D microarray chip was designed that is ideally suited to examine the effects of culture medium on CHO cell growth and specific mAb expression (Figure 1A). A commercially available 8-well chamber system was attached to the DataChip to allow the screening of a number of medium additives provided to the wells, each of which contained 48 separate microcultures (60 nl/spot, 200 cells/spot) in an alginate matrix. By bringing the two together, the effects of the medium composition and solution conditions on cell growth could be determined in the DataChip using simple live-dead staining. Figure 1B represents scanned images of portions of the cellular microarrays demonstrating increased green fluorescence from the calcein dye as a function of culture time for both host (CHO-H1) and recombinant (CHO-A1) cell lines in the non-proprietary medium (base conditions). The fluorescence intensity is quantified using GenePix Pro software. A calibration curve obtained after printing chips with varying cell densities (Supporting Information Figure S3) and assayed with live-dead stain immediately afterwards, was used to relate the fluorescence intensity to the number of cells in each spot. Figure 1C demonstrates that cell growth (left panel) and recombinant antibody production (right panel) in the recombinant antibody producing CHO-A1 cell line in the DataChip platform can be obtained.

The growth of the CHO cells in the 3D HT DataChip platform is limited by evaporation of the media and integrity of microcultures during prolonged incubation. The small volumes of medium used in the chip cultures (250 μl/well) led to significant media evaporation throughout the course of the experiments, which caused important deviations in nutrient and metabolite concentration with culture time. This issue was addressed by both measuring precise changes in media mass with time to account for the evaporation and preventing evaporation through increased humidity in the environment (Supporting Information Figure S4). In addition, brightfield microscopy images of the microcultures revealed some cell detachment from the alginate spots after 8 days of culture, whereas spot integrity was intact for previous samples (Supporting Information Figure S5). This is probably due to the alginate matrix deterioration, as well as cell over-growth at long incubation times. Thus, terminal batch experiments up to only 6 or 7 days are possible with the current microarray setup.

Application of the microarray DataChip for evaluating media components on cellular processes

The high-throughput DataChip platform was further utilized to evaluate impact of media additives (glucose, pyruvate, vitamins, non-essential amino acid, manganese, zinc and copper) on cellular processes, including recombinant antibody production (Figure 2 and Supporting Information Figure S6-S10). It is important to note that evaporation rates of media for this experiment were substantial, as depicted in Supporting Information Figure S11; however, the appropriate corrections in the measured concentrations were performed.

The effect of varying the concentration of glucose and pyruvate was examined, due to the importance of these compounds as carbon sources in glycolysis. The results show a significant decrease in cell growth with increasing glucose concentrations (Figure 2 and Supporting Information Figure S6). With small increases in glucose concentration, slight increases in titer were also seen with a maximum titer of ~22 μg/ml at 5.5 g/l of glucose (specific productivity ~27 pg/cell/d). However, at higher glucose concentrations (≥ 7 g/L), the overall titer was reduced, despite apparent increases in specific productivity due to the lower IVCD. These results are important since rigorous control of glucose concentrations is often necessary to minimize by-products, such as lactate and ammonia, and thus prevent media acidification. The addition of pyruvate led to a moderate increase in cell growth and a concomitant decrease in recombinant monoclonal antibody production with respect to the base media, which ultimately resulted in a decrease in productivity at high pyruvate concentrations, from roughly 14 pg/cell/d in base conditions (0.08 mg/ml pyruvate) to 9 pg/cell/d at pyruvate concentrations of 0.19 mg/ml (Figure 2 and Supporting Information Figure S6). In contrast, the addition of increasing concentrations of a MEM vitamin solution negatively impacted both cell growth and mAb production, resulting in extremely low productivities at the higher vitamin concentrations (~1.5 pg/cell/d) (Figure 2 and Supporting Information Figure S6). It was expected that MEM non-essential amino acids solution would enhance recombinant antibody production, since amino acids serve as the building blocks of proteins. These results show an increase in cell growth up to 2x concentrations of the amino acid solution that is offset by a similar increase in mAb concentration, resulting in a very small decrease in productivity (Supporting Information Figure S7). In addition, variations of Mn^{2+} (Supporting Information Figure S8), Cu^{2+} (Supporting Information Figure S9), and Zn^{2+} (Supporting Information Figure S10) on cell growth and productivity were analyzed. In all the chip platform experiments, growth data represents average (± standard error) of 48 technical replicates. The growth data does include the standard error, but it is small because of the large number of replicate cultures. Due to the miniaturization of the chip platform, the spent media from the 48 technical replicates were pooled to generate productivity data. Due to the fact that these experiments did not have biological replicates, it is difficult to discern if the changes in productivity observed are statistically significant.

In parallel, similar experiments were performed in conventional shake flasks comparing similar concentrations of pyruvate, MEM amino acids and Mn^{2+} as the 3D HT DataChip platform (Supporting Information Figure S12). In agreement with the Chip platform, the shake flask data showed that there was no significant differences in cell growth due to addition of various concentrations of pyruvate, MEM amino acids and Mn^{2+} to the base

media (Supporting Information Figure S12). Metabolite analysis of the spent media from samples obtained from shake flasks were conducted with BioProfile FLEX on cell-conditioned media from the shake flask experiments highlighted that the glutamate concentration used in the non-proprietary formulation was 20-fold lower than what is typical in the proprietary media formulation (data not shown). Glutamate is an essential amino acid and is an important source for production of glutamine [18]. Therefore, the 3D HT DataChip platform was utilized to evaluate effect of glutamate on cell growth and metabolism, and further crosscheck with the conventional shake flask platform. In addition, it was also observed that due to technical errors, there might be variability in preparation of the non-proprietary media (Table 1), for example minute changes in pH of initial base media influence cell growth and productivity and pH was adjusted at 7.0. Therefore, for all experiments, two 1L flasks of base media were prepared and sterilized at a time in parallel, that was kept in 4°C and used for experiments (within 2 months). In addition, our initial experiments showed that minute changes in the temperature, humidity and CO_2 levels of the incubator could impact our study. For consistency, all the experiments were performed in the same incubator, accommodating the shake flasks and 3D HT DataChips.

Glutamate addition and endogenous lactate consumption

The addition of 5 and 10 mM glutamate from the base condition of 0.3 mM led to a modest increase in cell growth in the 3D microarray DataChip platform (Figure 3A and 3B). Metabolite analysis on cell-conditioned media from the chip samples was performed using an YSI analyzer. Figure 3 demonstrates the effect of glutamate concentration on glucose consumption (Figure 3C), lactate formation (Figure 3E), and glutamate metabolism (Figure 3G). The data is helpful in illustrating the adequate preparation of the media as it concerns glucose and glutamate addition. The data also demonstrates that glucose is not depleted throughout the course of the 8-day experiment. An interesting increase in lactate concentration with increasing glutamate concentration was also observed.

The glutamate experiments were replicated in the shake flask platform (Figure 3B). The base media contained 0.3 mM glutamate. Addition of 5 mM and 10 mM glutamate demonstrated a modest increase in the cell growth in the CHO-A1 cells (Figure 3B). Figure 3 demonstrates effect of glutamate concentration on glucose consumption (Figure 3D), lactate formation (Figure 3F) and glutamate metabolism (Figure 3H) in the CHO-A1 cells. The trend of glucose consumption and glutamate concentrations in both DataChip (glucose, Figure 3C; glutamate, Figure 3G) and shake flasks (glucose, Figure 3D; glutamate, Figure 3H) were similar. The lactate production in the Datachip (Figure 3E) and shake flask (Figure 3F) were similar; however, a decline in lactate concentration on day 3 to day 4 in shake flask suggested lactate consumption for the highest glutamate concentration (glutamate concentration 10.3 mM, Figure 3F). Indeed, some difference in cellular bioprocesses, such as cell growth and mAb expression, might be reasonably expected in the two platforms. One of key differences between the two platforms is the cell density (10-fold difference in initially seeding density), which is expected to lead to varying degrees of nutrient availability and metabolite accumulation.

Lactate production leads to decrease in cell growth and productivity [22, 24]. Lactate consumption by cells occurs under optimal conditions, resulting in a metabolic shift [21, 22]. We observe that the GS/MSX CHO cells grown in base media supplemented with 10.3 mM glutamate exhibit a metabolic shift on day 3 to day 4 when cultivated in shake flasks, resulting in lactate consumption. It has been shown that specific changes in metabolic pathways lead to this lactate metabolic shift [22, 25, 26]. A potential usage of the microarray DataChip platform would be screening of metabolic pathways through an in-chip immunofluorescence assays [27].

Conclusion

High-throughput platforms have been a cost-efficient and rapid screening method for evaluating cell culture additives, including, screening of novel drugs and toxic chemicals in cell culture systems [4, 9, 10, 27]. The current study demonstrates that the DataChip platform has the potential to be utilized for evaluating impact of media additives, on cellular processes, such as, cell growth, metabolism and productivity. A more detailed metabolic flux analysis, as well as up/down regulation of key signaling pathways using the in-cell immunofluorescence assay developed for the chip, could also be performed [4]. Future work will be directed towards the glycosylation profiles of the glycoproteins produced on chip must be analyzed and compared to shake flask cultures. Analyses of *N*-glycans released by PNGaseF from glycoproteins secreted into the 250 μl should be well within the sensitivity possible using LC-MS analysis (Supporting Information Figure S2). Other experiments could involve factorial design of media additives for studying component interaction.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Abbreviations

CHO	Chinese hamster ovary
3D	three dimensional
HT	high throughput
LC-MS	liquid chromatography-mass spectrometry
IVCD	integrated viable cell density

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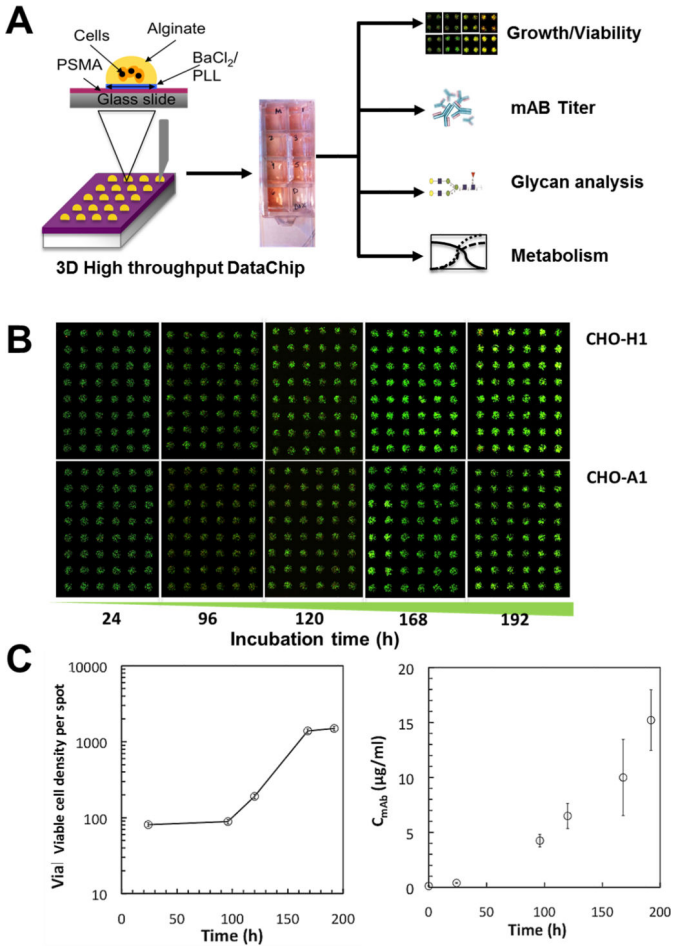

Figure 1.

Development of a three-dimensional high throughput DataChip microarray platform for growth of a recombinant antibody producing CHO –A1 cell. (A) Schematic diagram of the microscale platform approach for CHO cell growth optimization and product analysis. (B) A commercially available 8-well chamber system was attached to the DataChip to allow the screening of a number of medium additives provided to the wells, each of which contained 48 separate microcultures (60 nl/spot, 200 cells/spot) in an alginate matrix. Live dead assay analysis for growth of host CHO-H1 and recombinant CHO-A1 cells in DataChip platform using calcein dye. The fluorescence intensity is quantified using GenePix Pro software. (C) Analysis of (i) growth and (ii) recombinant monoclonal antibody production in CHO-A1 cell line on DataChips. Cell growth was determined using cell viability assay. Recombinant monoclonal antibody production was determined with MSD competition assay. Data represents the mean ± SE of an experiment with 190 replicate microcultures for the live dead assay, and two replicate conditioned media samples for antibody production.

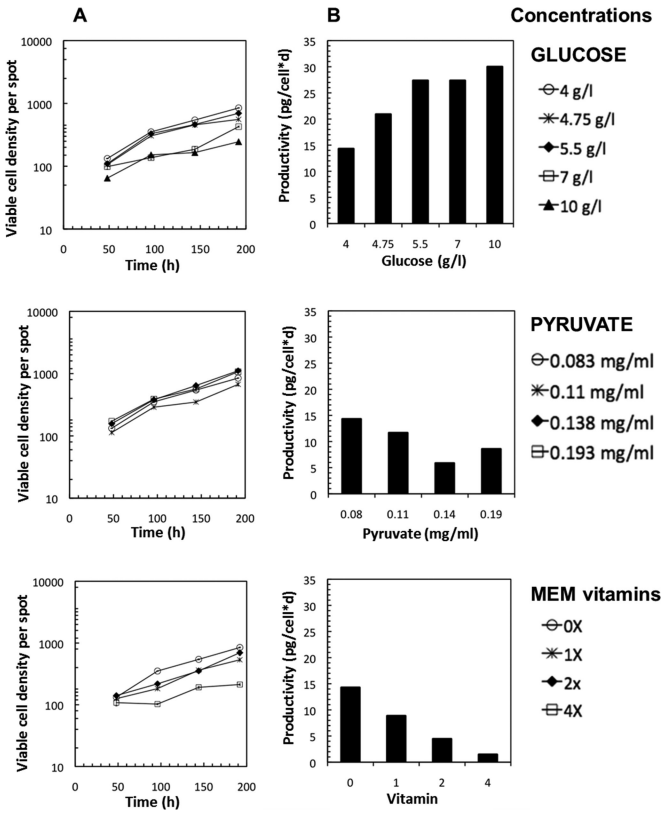

Figure 2.

Application of the DataChip for media optimization. Changes in (A) cell growth, and (B) productivity in response to variations in glucose (4, 4.8, 5.5, 7 and 10 g/l), pyruvate (0.08, 0.1, 0.14 and 0.19 mg/ml), and MEM vitamins (0, 1x, 2x and 4x to 2x concentration of the solution). Cell growth was determined using cell viability assay. Recombinant monoclonal antibody production was determined with MSD competition assay. Cell growth analysis data represents the mean ± SE of an experiment with 48 replicate microcultures and pooled spent media from the 48 microcultures for antibody productivity analysis.

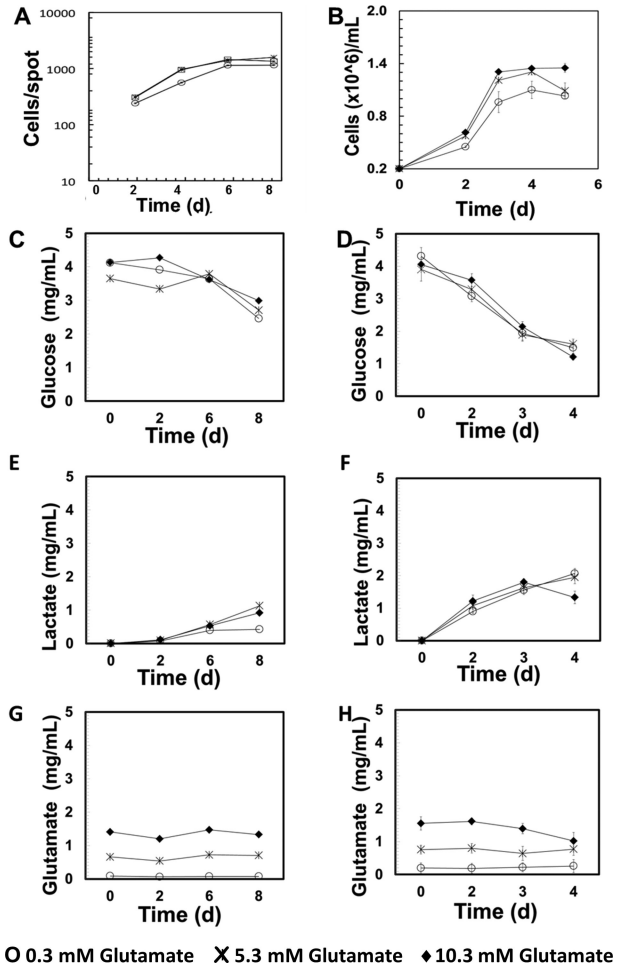

Figure 3.

Impact of glutamate concentration on cell growth and metabolism in Datachip and shake flask platforms. Changes in (A) cell growth in DataChip platform, (B) cell growth in shake flask platform, (C) glucose metabolism DataChip platform, (D) glucose metabolism shake flask platform, (E) lactate production DataChip platform, (F) lactate production shake flask platform, (G) glutamate concentration DataChip platform and, (H) glutamate concentration shake flask platform. Cell growth in DataChip platform was determined using cell viability assay. Cell growth in shake flask platform was determined by manual cell counting using trypan blue, according to the standard operating protocol provided by J&J, Janssen Biotech Metabolite analysis on cell-conditioned media collected from the chip samples and shake flask samples were performed using an YSI analyzer. DataChip data represents the mean ± SE of an experiment with 96 replicate microcultures and pooled spent media from the 96 microcultures for metabolite analysis. Shake flask data represents the mean ± standard deviation, where, n=2 independent experiments.

Table 1

Non-proprietary medium formulation[a)]

Base	**Amounts**
Minimum Essential Medium Eagle, Alpha modification, with ribonucleosides, deoxyribonucleosides and sodium bicarbonate, without L-glutamine, liquid, sterile-filtered, suitable for cell culture (Sigma-Aldrich)	500 ml
Dulbecco's Modified Eagle's Medium/Nutrient Mixture F-12 Ham, With 15 mM HEPES and sodium bicarbonate, without L-glutamine and phenol red, liquid, sterile-filtered, suitable for cell culture (Sigma-Aldrich)	500 ml
Supplements	**Amounts**
Glucose	2 g
Pluronic F-68	4 g
$NaHCO_3$	1.3 g
Chelated Ferric Citrate	0.3 mM
Ethanolamine	0.04 mM
Bacto Tryptone	2.5 g
Glutamine	292 mg
Putrescine	0.1 mg
$MnSO_4.H_20$	0.2 μg
Na_2SeO_3	2 μg
$(NH_4)_6Mo_7O_{24}.4H_20$	0.01 mg
Vitamin B6	0.1 mg
NH_4VO_3	1 μg
Insulin	5 mg
Linoleic Acid	0.1 mg
Glutamax	Varies as per cell line
Hypoxanthine-thymidine	Varies as per cell line
MSX (methionine sulphoximine)	Varies as per cell line
Amino acids	**Amounts**
L-Proline	20 μM
L-Tyrosine	20 μM
L-Serine	20 μM
L-Histidine	20 μM

[a)] Per 1 liter of medium