Seminar on Research Findings Series No. 2/2009. Research Management Center, International Islamic University Malaysia

Research Management Centre
International Islamic University Malaysia

OPTIMIZATION OF MODIFIED-MEDIA CONTAINING SERUM FOR BETTER RIN-5F AND BRIN-BD11 CELLS MONOLAYER ATTACHMENT, GROWTH AND INSULIN SECRETION.

By Prof. Dr. Mohamed Ismail Abdul Karim
Department of Biotechnology Engineering, Kulliyyah of Engineering, International Islamic University Malaysia, P.O.Box 10, 50728 Kuala Lumpur, Malaysia.

Abstract:

A study was undertaken to observe the effect of D-glucose and L-glutamine and the combination of both nutrients at various concentrations towards the growth and insulin production by BRIN-BD11 cells. It was found that at a final concentration of 4 g/L D-glucose and 0.3 g/L L-glutamine in RPMI-1640 media gave maximum cell growth and insulin production of 1.75 x 10^6 cells/ml from 2.5 x 10^5 cells/ml and 100.77 µg/L, respectively. The result of this study indicates that D-glucose and L-glutamine promote good cell growth and insulin secretion of BRIN-BD11 cells as a step towards mass propagation of transplantable insulinoma cells and insulin production.

Key words: BRIN-BD11, D-glucose, L-glutamine, Insulin

I. INTRODUCTION

Insulin derived from genetically engineered bacteria has been known to dominate the global insulin market for diabetic treatment in this century. However, apart from improving insulin production, the cell transplantation technology has

recently gained much interest by many scientists in making the diabetic treatment permanent. Therefore, an anormous supply of healthy cells are needed for the theraphy. The cells may derived from patients either in the form of primary isolated islet cells or cells developed from the stem cells. However, the short lifespan, small number of viable cells and low secretion of insulin posses by these cells were known to limit the understanding of their metabolism and functions.

There are a number of insulinoma cell lines that have been developed through tissue cell engineering in order to overcome these limitations [1]. However, most of these insulinoma cell lines performed moderate to unresponsive towards certain concentrations of glucose in stimulating insulin secretory and drove the development of new hybrid insulinoma cell, the BRIN-BD11 cells [1]. The cells has gained much attention as a model cell in insulin related study due to its immortality and normal features of insulin biosynthesis and secretion from the normal pancreatic β-cell [1].

The functions and effects of nutrients towards mammalian cell attachment, growth and propagation are important to avoid cell toxicity and apoptosis in order to stimulate maximum cell growth in media. Glucose and L-glutamine are two most important compounds that are normally formulated in the mammalian cell growth media such as the DMEM/Ham's nutrients mixture F12, RPMI 1640 and serum-free hybridoma media at various concentrations [2].

Glucose is a hexose sugar act as fuel to provide energy in cell metabolic pathways for cell to function [2]. D-glucose was found to stimulate insulin more than D-mannose and unresponsive with other hexose sugars by BRIN-BD11 cells [1].

The L-glutamine helps in synthesizing large amount of proteins and nucleic acids. Apart from that, it supports high energy to the rapidly dividing cell and cells that use glucose inefficiently [3].

Our efforts in this study is to identify the optimum concentration of glucose and L-glutamine needed for BRIN cells to attach, grow and propagate towards the mass production of cells in tissue culture flask that could be used for future in bioreactor propagation for better insulin production.

Objective: To improve growth media formulation for growing of BRIN-BD11 cell and the secretion of insulin.

II. MATERIALS AND METHODS

A. Cell culture

BRIN-BD11 cells were generously provided by Dr. Muhajir Hamid from Department of Microbiology, Faculty of Biotechnology and Biomelecular Sciences, Universiti Putra Malaysia. The cells were maintained in 25 cm^2 T-flask (Orange Scientific, E.U.) containing 4.5 g/L D-glucose (Calbiochem, Germany) and 0.3 g/L L-glutamine

(Amresco,USA) supplemented inRPMI-1640 growth media from Celgro, USA (2 g/L glucose without L-glutamine) (formulation recommended by ATCC [4] for epithelial cells), 10% fetal bovine serum, 100 U/ml penicillin and 0.1g/l streptomycin (both were from PAA, Austria) at pH 7.4 were then incubated in a CO_2 incubator (Galaxy, RS Biotech, Germany) at 37^0C with 5% CO_2 atmosphere.

B. Preparation of cells

The cells were detached by Accutase purchased from Innovative Cell Technologies, Inc., USA and was inoculated into a 175 cm^2 vented-cap tissue culture flask containing the same growth media for maintaining the cell culture (same growth media as in section A) until 80% confluence. Then, 1 x 10^6 cell/ml was collected and washed three times with RPMI media (without D-glucose and L-glutamine) by 1000 rpm centrifugation (Centrifugal 5804 R, Eppendorf, Germany) at 4^0C for 15 minutes for each cycle.

C. Culture media

Excess amount of D-glucose and L-glutamine was added into the RPMI-1640 media (supplemented with 10% FBS and 100 U/ml penicillin and 0.1g/l streptomycin) to a different final concentrations of D-glucose (2, 4 and 6 g/L) and L-glutamine (0, 0.3 and 0.6 g/L) and the combination of both D-glucose (D) and L-glutamine (L) (2(D) + 0.3 (L), 2(D) + 0.6 (L), 4(D) + 0.3 (L), 4(D) + 0.6 (L), 6(D) + 0.3(L), 6(D) + 0.6(L) all in g/L). pH of the all media was adjusted to 7.4.

D. Effect of Glucose and L-glutamine on cell culture

Cells collected from section C were diluted into 2.5 x 10^5 cells/ml of prepared culture media. Then, 10 ml of the suspension was cultured into a 150 mm tissue culture dish (Orange Scientific, E.U.) and was incubated in a CO_2 incubator at 37^0C with 5% CO_2 atmosphere. Daily sampling was done for 8 days. Media replacement was conducted when pH drop to 7 [5].

E. Sample analysis

pH of the suspension was measured by using the pH probe and meter (Toledo, Switzerland), viable and non-viable cell number of both attached and suspension cells were counted using the Neubauer improved haemocytometer (Hirchmann EM Technocolour, England) under light microscope (Olympus CX21, China). Glucose and L-glutamine concentrations of the suspension were measured by using the biochemical analyzer (YSI, USA). Enzyme immunoassay high range rat insulin ELISA (Mercodia, Sweden) was used to measure the insulin concentrations detected by ELISA reader (BIO-TEK Instruments, Canada).

III, RESULTS

A. Cell growth

In this study, the optimum concentration of glucose and L-glutamine in stimulating the growth of BRIN-BD11 hybrid insulinoma cell and insulin production in RPMI-1640 media supplemented with various concentrations of D-glucose and L-glutamine was observed.

Figure 1, shows the growth profile of BRIN-BD11 cell culture in RPMI-1640 media containing different concentration of D-glucose, L-glutamine and the combination of both D-glucose and L-glutamine. The BRIN-BD11 culture manage to grow in RPMI-1640 media containing low concentration of glucose and without L-glutamine reaching a cell density of 3.56×10^5 cells/ml at day 3 until it drop to the lowest value of 1.97×10^4 cells/ml at day 6.

Culture in media consisting 4 g/L D-glucose and 0.3 g/L L-glutamine reach the highest peak of 1.75×10^6 cells/ml at day 7 from a starting culture number of 2.5×10^5 cells/ml. The second highest point was at the same day with cell density of 1.51×10^6 cells/ml in media supplemented with 6 g/L glucose and 0.3 g/L L-glutamine. Whereas, the third highest peak was at day 6 with 1.17×10^6 cells/ml in media containing 6 g/L glucose with 0.6 g/L L-glutamine.

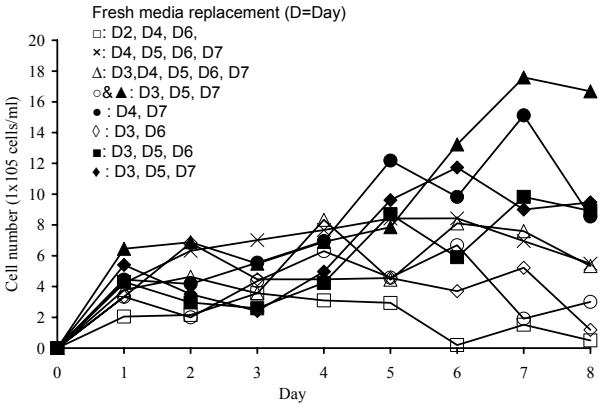


Fig. 1 Attached viable cells in RPMI-1640 media supplemented with (□) 2 g/L D-glucose, (○) 2 g/L D-glucose with 0.3 g/L L-glutamine, (◊) 2 g/L D-glucose with 0.6 g/L L-glutamine, (x) 4 g/L D-glucose, (▲) 4 g/L D-glucose with 0.3 g/L L-glutamine, (■) 4 g/L D-glutamine with 0.6 g/L L-glutamine, (Δ) 6 g/L D-glucose, (●) 6 g/L D-glucose with 0.3 g/L L-glutamine, (♦) 6 g/L D-glucose with 0.6 g/L L-glutamine

B. Insulin production

Insulin produced in RPMI-1640 media containing 2, 4 and 6 g/L D-glucose without L-glutamine was able to produce as high as 0.92, 2.96 and 2.3 µg/L of insulin, respectively. The highest insulin produced was at day 2 with 100.77 µg/L insulin in RPMI-1640 supplemented with 4g/L D-glucose and 0.3 g/L L-glutamine. Meanwhile, the second highest was 27.02 µg/L insulin produced after 24 hours in RPMI-1640 supplemented

with 2 g/L D-glucose and 0.3 g/L L-glutamine. The third highest peak appeared later at day 6 with 5.08 µg/L insulin produced in RPMI-1640 supplemented with 4 g/L D-glucose and 0.6 g/L L-glutamine (Fig. 2).

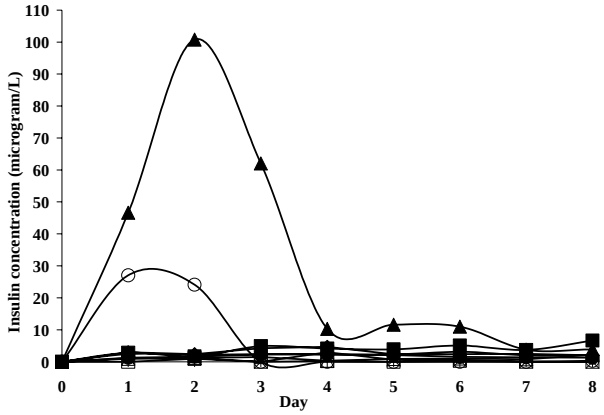
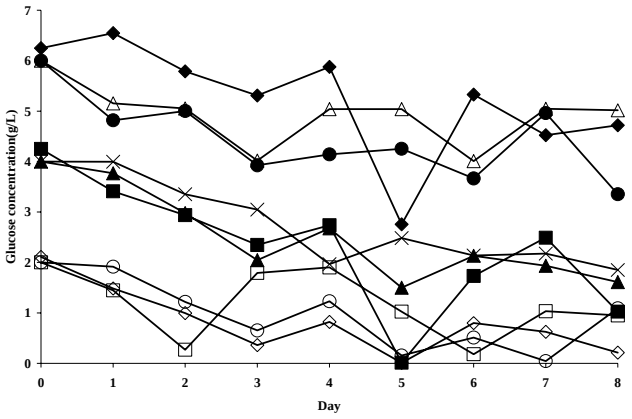


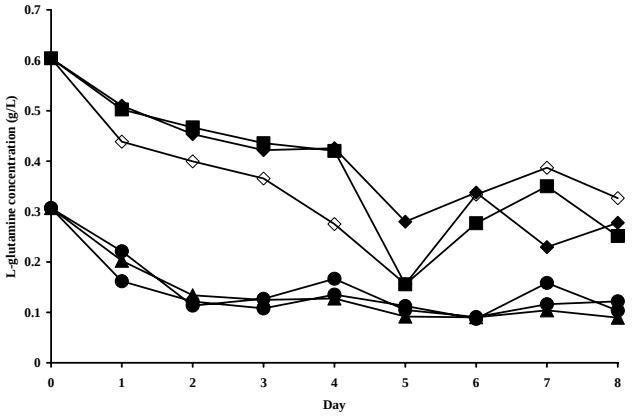
Fig. 2 Insulin produced by BRIN-BD11 cells in RPMI-1640 media supplemented with (□) 2 g/L D-glucose, (○) 2 g/L D-glucose with 0.3 g/L L-glutamine, (◊) 2 g/L D-glucose with 0.6 g/L L-glutamine, (x) 4 g/L D-glucose, (▲) 4 g/L D-glucose with 0.3 g/L L-glutamine, (■) 4 g/L D-glutamine with 0.6 g/L L-glutamine, (Δ) 6 g/L D-glucose, (●) 6 g/L D-glucose with 0.3 g/L L-glutamine, (♦) 6 g/L D-glucose with 0.6 g/L L-glutamine

C. D-glucose and L-glutamine uptake

It was found that 25% and 66.7% of D-glucose and L-glutamine, respectively was consumed at the point where insulin was secreted at the highest insulin peak (100.77 µg/L insulin) when culture was exposed in RPMI-1640 media supplemented with different concentrations of D-glucose and L-glutamine (figure 3 (a) and (b)). Meanwhile, 19% and 46.7% of D-glucose and L-glutamine, respectively were consumed at the second highest insulin peak (27.02 µg/L insulin). At the third highest insulin peak (5.08 µg/L insulin), 16.7% and 43% of D-glucose and L-glutamine, respectively was consumed by the BRIN-BD11 culture.



(a)



(b)

Fig. 3 D-glucose (a) and L-glutamine (b) consumptions by BRIN-BD11 in RPMI-1640 media supplemented with (□) 2 g/L D-glucose, (○) 2 g/L D-glucose with 0.3 g/L L-glutamine, (◊) 2 g/L D-glucose with 0.6 g/L L-glutamine, (x) 4 g/L D-glucose, (▲) 4 g/L D-glucose with 0.3 g/L L-glutamine, (■) 4 g/L D-glutamine with 0.6 g/L L-glutamine, (Δ) 6 g/L D-glucose, (●) 6 g/L D-glucose with 0.3 g/L L-glutamine, (♦) 6 g/L D-glucose with 0.6 g/L L-glutamine

IV.DISCUSSION

This study was conducted to identify the optimum concentration of D-glucose and L-glutamine to improve the BRIN-BD11 cell growth and insulin production. The RPMI-1640 growth media was claimed to be the most suitable available commercial growth media for the BRIN-BD11 cells as it gave the best insulin release [6]. The commercial media available are normally formulated with glucose and glutamine at concentrations between 1 to 10 g/L and 0.073 to 1.46 g/L, respectively [2]. The requirement of these nutrients may vary from one cell line to the other.

The BRIN-BD11 cells responsive towards sugars, amino acids, neurotransmitters, peptide hormones and drugs after the cells was cultured in RPMI-1640 media have shown good secretion of insulin [6].

From the experiment, glucose and L-glutamine was heavily utilized in maintaining cell growth insulin production. Brennan, L. *et al*., (2002) [7] and Dixon, G. *et al*., (2003) [3] had claimed that glucose is consumed at high rates by primary rat islet cell and BRIN-BD11 cell, and is closely related with in insulin secretion [8].

The BRIN-BD11 culture manages to grow and survive even at a low concentration of glucose (2g/L) and without supplementation of L-glutamine in RPMI-1640 growth media. It was reported that glucose concentration above 0.64 g/L may trigger insulin production [9] and glucose involves in insulin release through the glucose phosphorylation process which depolarizes the cell membranes and activates exocytosis process which release insulin from β-cell [10]. However, insufficient glucose and glutamine supply will cause cell death and exposure to high glucose concentration will impair β-cell functions and reduce the cell sensitivity to secrete insulin. This situation has been observed through this study. Therefore, the right combination of both D-glucose and L-glutamine concentration is crucial for β-cell to perform.

The BRIN-BD11 culture grew and produced insulin best in media supplemented with 4 g/L D-glucose and 0.3 g/L L-glutamine. In a study by McClenaghan, N. H. (1996) [1], BRIN-BD11 cells showed good response towards glucose concentration ranges between 0.25 and 6.05 g/L within 20 min. Sufficient presence of D-glucose and L-glutamine in the media, when pH drop to 7, fresh media replacement helps to keep the nutrients available for the cells to grow and secrete insulin to the maximum.

Glutamine is an essential nutrient that is heavily utilized by in cell proliferation [11]. It also beneficial in dealing with metabolic stress as it may enhance cell survival [12]. Glutamine had to be present in 10 to 100 fold excess than any other amino acid in culture and could not be replaced by glutamic acid or glucose [13]. Glutamine has been reported to enhance glucose-stimulated insulin secretion from pancreatic β-cell, however, does not promote insulin secretion by itself due to tight regulation of glutamate dehydrogenase activity [14]. The presence of glutamine in the β-cell pathway cycle enhances glucose oxidation which later induces insulin exocytosis [13].

III. CONCLUSIONS

It can be concluded that good growth (1.75 x 10^6 cells/ml from 2.5 x 10^5 cells/m) and insulin production (100.77 µg/L) of BRIN-BD11 cells can be achieved together in RPMI-1640 media supplemented with D-glucose and L-glutamine at concentrations of 4 g/L and 0.3 g/L, respectively.

IV. ACKNOWLEDGEMENT

The authors wish to thank the International Islamic University Malaysia and the Malaysian government for providing the funds for this research.

VI. REFERENCES

1. McClenaghan N H, Barnett C R et al. (1996) Characterization of novel glucose-responsive insulin-secreting cell line, BRIN-BD11, produced by electrofusion. Diabetes 45:1132-1140
2. Sigma-Aldrich at http://www.sigmaaldrich.com
3. Dixon G, Nolan J et al. (2003) A coparative study of amino acid consumption by rat islet cells and the clonal beta-cell line BRIN-BD11- the functional significance of L-alanine. Journal of Endocrinology 179:447-454
4. Animal Tissue Culture Collection (ATCC) at http://www.atcc.com
5. Freshney R I (2000) Culture of animal cells: A manual of basic technique. Wiley-Liss, USA
6. Hamid M, McCluskey J T et al. (2001) Comparative functional study of clonal insulin-secreting cells cultured in five commercially available tissue culture media. Cell Transplantation 10:153-159
7. Brennan L, Shine A et al. (2002) A nuclear magnetic resonance-based demonstration of L-alanine metabolism and L-alanine-enhanced glucose metabolism in a clonal pancreatic β-cell line. Diabetes 51:1714-1721
8. Henquin J C (2000) Triggering and amplifying pathways of regulation of insulin secretion by glucose. Diabetes 49:1751-1760
9. Ashuroft S J H and Randle P J (1968) Glucose phosphorylation in mouse pancreatic islets
10. Wollheim C B and Maechler P (2004) β-cell biology of insulin secretion. *In* International textbook of diabetes mellitus. DeFronzo R A, Ferrannini E et al. (2004) John Wiley and Sons Ltd., England

11. Reitzer L J, Wise B M et al. (1979) Evidence that glutamine, not sugar, is the major energy source of cultured HeLa cells. J Biol Chem 254:2669-2676
12. Klimberg V S and Souba W W (1990) The importance of intestinal glutamine metabolism in maintaining a healthy gastrointestinal tract and supporting the body's response to injury and illness. Surg Ann 22:61-76
13. Newholme P, Lima M M R et al. (2003) Glutamine and glutamate as vital metabolites. Braz J Med Biol Res 36(2): 153-163
14. Gao Z Y, Najafi H et al. (1999) Glucose regulation of glutaminolysis and its role in insulin secretion. Diabetes 48:1535-1542