Understanding Metabolic Needs of EB14 Cells in Culture

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Abstract: EB_{14} is a genetically stable diploid cell line derived from chicken embryonic stem cells. EB_{14} cells demonstrate great potential in virus and recombinant protein production for prophylactic and therapeutic purposes. The present study aims at identifying metabolic needs of the EB_{14} cells so that specific nutrients can be provided adequately and in a timely manner. EB_{14} cells exhibit unique metabolic patterns in various serum-containing and serum-free media, and are highly sensitive to nutritional supply and waste accumulation in culture. Typically, EB_{14} cells require frequent replenishment of culture media to stay viable. The ratio of aerobic to anaerobic metabolism was calculated based on carbon and nitrogen energy source consumption rates in EB_{14} cells. As a result, optimal media formulations and culture processes were developed to support high viable cell growth supporting recombinant and attenuated viral vaccine production. Chemical and cellular analysis of EB_{14} cell cultures with specific viable cell density (VCD), culture longevity and viral productivity will be discussed.

Key words: Chicken Embryonic Stem Cells, EB_{14}, Media Development, Metabolic Analysis, Influenza Vaccine.

1. INTRODUCTION

The current flu vaccine manufacturing process issues have caught the world's attention and revealed concerns about the ability to provide an adequate supply of vaccines. Currently the flu vaccine is produced primarily

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in chicken embryonic fibroblasts (CEFs). Processes based on CEFs are highly dependent on the tedious process of isolating and culturing CEFs from chicken eggs. EB_{14} cells provide a brand new cell culture platform to the vaccine industry with immediate benefits. This avian stem cell line was first successfully isolated in long-term culture by Vivalis (Nantes, France). JRH Biosciences and Vivalis scientists are the first team to develop a serum-free media and process to support high cell density and viral production in EB_{14} cells. More interestingly, JRH and Vivalis scientists investigated EB_{14} cell's energy expenditure and metabolic pathway control through media nutrient optimization.

2. MATERIALS AND METHODS

2.1 Cells and Culture Media

Stock cultures of EB_{14} (Nantes, France) were maintained in a 37°C, 7.5% CO_2 humidified incubator in EX-CELL™ 60947, 65318, 65319, 65320, and 65126 (JRH Biosciences, Inc., Lenexa, KS) and competitor Medium X. The Influenza A/New Caledonia/20/90 (H1N1) was obtained from the Centers for Disease Control and Prevention (CDC). Madin Darby Canine Kidney (MDCK) cell line is from American Type Culture Collection (ATCC CCL-34).

2.2 Process Development

Bioreactors (Applikon® Biotechnology, Sciedam, Holland) were seeded at 0.2e6 cells/mL in EX-CELL™ 60947 and Medium X. Dissolved oxygen (DO) and pH were monitored online and controlled using air, O_2, N_2, CO_2, and 7.5% $NaHCO_3$, respectively.

2.3 Viral Infection

Twenty four hours prior to infection, EB_{14} cells were seeded at 0.4e6 cells/mL in 125mL Erlenmeyer flasks. Cells were inoculated with a multiplicity of infection (MOI) of 0.01 of Influenza A/New Caledonia and allowed to adsorb for 1 hour at 34°C. After 1 hour, additional media was added and the cultures were incubated at 34°C for 5 to 6 days. Trypsin was added at 3μg/mL each day while sampling cultures.

2.4 Western Blot Analysis

Ten microliters of supernatant from infected cells were used for a western blot using WesternBreeze Chromogenic Immunodetection Kit

(Invitrogen). Hemagglutinin (HA) Monoclonal Antibody (QED Bioscience Catalog# 20301).

2.5 Analytical Methods

Cell density and viability was determined by Cedex (Innovatis, Bielefeld, Germany) and by trypan blue exclusion method with hemacytometer. Metabolites dynamics was monitored offline with the BioProfile® 100 (Nova Biomedical Corporation, Waltham, MA).

3. RESULTS AND DISCUSSIONS

EB_{14} cells grew significantly higher in EX-CELL™ 60947 to greater longevity as compared to in Medium X (data not shown). EB14 cells in EX-CELL™ 60947 reached a maximum cell density of approximately 3e6 cells/ml on Day 2 and maintained this density through the Day 4 whereas rapidly declined in Medium X on day two. A similar pattern was reproduced in bioreactor runs (data not shown).

Metabolite analysis indicated the rate of lactate and ammonia production was much higher for the competitor Medium X than all EX-CELL™ 60947 formulations. However, the rate of glucose and glutamine consumption remained comparable among all formulations tested.

Western blot analysis indicates that EX-CELL™ 60947 supports the production of Influenza A/New Caledonia, as demonstrated by the presence of HA0 and HA2 (data not shown).

4. CONCLUSIONS

We conclude, thus, 1) EX-CELL™ 60947 is a serum-free, animal component free medium that supports higher cell densities, improved viabilities and greater culture longevity in comparison with Medium X; 2) Optimized EX-CELL™ 60947 supports Influenza Virus production.