In vitro expansion of embryonic stem cells in a fibrous bed bioreactor

Anli Ouyang, Shang-Tian Yang

Department of Chemical engineering, The Ohio State University, 140 West 19th Ave. Columbus, OH, 43210

Introduction: ES cells are considered a potential alternative source of cell therapy and tissue transplantation [1]. However, spontaneous differentiation often happens when embryonic stem cell is cultured *in vitro* [2]. When first isolated, it was believed that having murine embryonic fibroblasts as the feeder cell was essential for mouse ES cell maintenance [3]. However, if ES cells are used to treat human or veterinary patients, the feeder cells are the potential contamination source when harvesting ES cells in traditional 2-D culture. What is needed is the large scale expansion of undifferentiated embryonic stem cells without these feeder cells Thereby, it will supply enough amounts of ES cells for stem cell therapy. Most of current ES cell expansion works were done in 2-D culture system. This needs gelatin pre-coated surface and frequent passages to maintain their undifferentiated stage [4]. These labor intensive and time consuming work could not meet the projected high market demand. In this study, we tried to find an economic and scalable way to produce undifferentiated ES cells by replacing LIF with condition medium from STO cell culture and providing 3-D culture environment.

Experiment: ES D3 cells (CRL-1934) obtained from ATCC were maintained on gelatin pre-coated T-flasks. After STO cell reached 80% confluent in 75 cm^2 T-flask, change fresh medium and continued the culture for 4 days. Supernatant was collected for later use. Condition medium for 3-D long term culture was produced by following method. 3~5 millions STO cells were inoculated into a 250 ml spinner flask packed with PET scaffold with working volume as 110 ml. Changing medium at 6^{th} day and continuing culture for another 4 days. Supernatant was collected. Before use, glucose, FBS, non-essential amino acid, L-glutamine, β-mercaptoethanol, antibiotics were added to their final concentrations as normal ES cell growth medium. ES cells were also cultivated in spinner flask and a perfusion fibrous bed bioreactor to improve their growth as well as inhibit their spontaneous differentiation.

Results: In this study, a 3-D culturing method was developed for expansion of undifferentiated murine ES cells. Cell growth and differentiation kinetics in T-flasks (2-D cultures) and PET scaffolds (3-D cultures) were studied and compared. In general, surface coating with gelatin was critical to cell attachment and proliferation for the 2-D culture, but not for the 3-D culture. ES cells' attachment kinetics in 3-D matrix was studied and it was found that the process was follow first order kinetics and the ES cells could attach to the matrix faster than CHO cells. Murine ES cells maintained their pluripotency as well as proliferation potential in 3-D PET scaffolds for more than 9 days. For cells cultured in the 3-D matrix with passage every 2 days, 98% of cells after 9 days were SSEA-1 positive, indicating the undifferentiated state. To reduce the medium cost, the expensive medium component, LIF, usually required for maintaining ES cells in the undifferentiated state was replaced with conditioned medium from STO cells. In 7 passages, ES cells grown in the conditioned medium had similar expansion fold and SSEA-1 and specific ALP expression levels as those from cells grown in the LIF containing medium. However, there was notable down regulation of these two markers in the long-term cultures (21 days). Our results showed that high initial lactic acid concentration and rapid accumulation during the culture in the growth media had great effect on ES cells' differentiation. Therefore,

dynamic culture method was employed to improve oxygen transfer rate and therefore to lower lactic acid production rate. What's more, the dilution of condition medium by fresh medium could also lower the initial lactic acid concentration and we found 50% of condition medium could well sustain ES cells' growth without notable spontaneous differentiation in the long term culture. A perfusion fibrous bed bioreactor (FBB) was developed for production of undifferentiated ES cells. ES cells were cultured in the FBB for 18 days without passage. The results showed high SSEA-1, Oct-4 expression level and continuous expansion during the entire culturing period, indicating the feasibility of achieving both a high expansion fold and maintaining undifferentiated state in the long-term culture using the bioreactor.

Discussion: It was found that ES cells could grow well in 3-D PET scaffold without gelatin coating, which cut the cost of the process. The reason why 3-D coating was not better than 3-D no coating was probably that coating deteriorated the 3-D structure of scaffold. Our 3-D culture method had more advantages than this. ES cells can grow in the scaffold for a pretty long time (15 days) without passage and they didn't lose their pluripotency. And the PET scaffold is much cheaper than T flask. LIF was reported to be necessary to maintain ES cells undifferentiated stage in feeder cell free culture system. However, LIF is expensive and takes more than 50% of the medium cost. The ES cell production process will be more profitable if the cheap replacement of LIF can be found. STO cells were used as feeder cell to support undifferentiated ES cell growth. They are believed to secrete some unknown cytokines which are essential to ES cells. . However, it was found condition medium from 3-D culture can't function well in ES cell's long term culture. Only 58% of ES cells kept undifferentiated after two weeks culture in condition medium. Why is there a difference between two cases? It was found that the initial lactic acid concentration in condition medium from 3-D culture is much higher (2.2~2.4 g/l) than 2-D. Mixing could partially solve this problem by increasing oxygen transfer rate. Frequently changing medium could also work. So dynamic and perfusion culture were applied. They both improved ES cells growth with keeping their pluripotency. So, our in vitro perfusion fibrous bed bioreactor system is suitable for the mass production of undifferentiated ES cells.

Reference:

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