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International Seminar and 2nd Congress of SEAVSA

INCREASING ANIMAL PRODUCTION THROUGH ZOONOSES AND REPRODUCTIVE DISORDER HANDLING, AND THE IMPLEMENTATION OF BIOTECHNOLOGY

Surabaya, 21-22 June 2011
Royal Ballroom, JW Mariott Hotel
Surabaya - Indonesia

Editors:
Mustofa Helmi Effendi (Indonesia)
Rahayu Ernawati (Indonesia)
Muhammad Yunus (Indonesia)
Jean-Francois CHARY (French)
Achariya Sailasuta (Thailand)
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CONTENTS

FULL PAPER

CELL CULTURE: FUTURE PROSPECT

Bambang Sektiari Lukiswanto
Faculty of Veterinary Medicine Universitas Airlangga

The cell culture was initially by Ross Harrison in 1907 who demonstrated the frog embryo growth in vitro. This technology showed the important role since the specific individual cells and the first continuous cell lines were developed and the cells were not only kept alive by cryopreservation technology but also they grew as they would do in vivo after thawing and reculturing. The specific requirements of the differences cell types like local micro-environment, local growth factors, extra cellular matrix, cell-cell interactions, circulating proteins, cytokines and hormones were being developed for the growth of specific cell types desired. The advances in cell culture techniques enable researchers to create at least partially model complex interactions, drug testing and investigate toxic effects, further more incorporating cells from several different tissues in the culture offer the opportunity to create the interactions among separate biological subsystems in vitro. The major benefit of cell culture technology utilisation is not only greatly felt in the biomedical, production and reproduction areas but also in the biopharmaceutical development. The future prospective of cell culture technology among others perfect cell culture system for the preparation of autologous cell lines, the processing of xenogenic cell lines for cellular implants, gene therapy, artificial organ, and three-dimensional cell cultures development.

Key words: Cell culture, in vitro, spesific requirement, perfect cell culture system

Cell Culture: Future Prospect

Bambang Sektiari L.
Clinical Veterinary Department, Veterinary Medicine Faculty
Airlangga University
Email : bamsekti@yahoo.com

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Introduction

Cell culture was initiated by Ross Harrison (1907) which showed the growth of frog embryos in vitro, He also proved that the nerve axons can be formed from the development of neuronal cell body. Enders *et al.* (1949) observed that non-neuronal cell cultures of primary monkey kidney cells can proliferate in vitro so it can be used to produce the polio virus, this discovery opens the way for large scale production of polio vaccines using animal cell cultures. Nicolas (1978) utilized fibroblast cell culture to produce rabies vaccines. In the early eighties, Montagnon (1984) developed Vero cells for commercial production of inactivated polio vaccine. Since 1995, starting the usage of continuous cell line cultures for human biological products. Further development was the discovery of various culture-based cell technology for heterologous protein expression, recombinant CHO-technology,

the amplification and selection markers and glutamine synthetase (GS) and the development of baculovirus cell technology, NS0-GS recombinant technology today has grown rapidly and allows manufactured monoclonal antibody on a large scale reactor (Merten, 2006) .

Source and principle of Cell Culture

The cell culture technique can be evaluated by certain parameters, ie cell morphology, growth rate, growth efficiency, and a special function held by cells. The most important element in the cell culture technique is the ability to provide a sufficient numbers of cells and maintain theirs phenotypes and to carry out the necessary biological functions. the cells must be able to produce extracellular matrix in the appropriate organization, interact with the neighboring cells and the surrounding tissue and secrete cytokines and other signaling molecules.

There are several sources of cells used today. i.e., primary cells, and cell lines. Primary cells are cells harvested directly from a part of the tissues or organ fragments whether mechanical or enzymatically, for example, endothelial cell removed form blood vessels, osteoblasts harvested from the femoral heads. While the cell lines is a cell population derived from a primary cell cultures at the first sub culture. The advantage of using primary cells is associated with immunological compatibility but some cell type can de-defferentiate during ex vivo harvesting and reveal the in appropriate phenotypes (for example, articular chondrocytes in culture often produce fibrocartilage as opposed to hyaline cartilage). Although primary cells still frequently used but the growth rate and the results tend to be low. This limitation has prompted researchers to develop alternative sources of cells for tissue engineering strategies and continuous cell lines as a solution to some problems encountered by using primary cells.

The grow cells in vitro must mimic in vivo condition, so a various culture media have been developed, these are: DMEM, BME, EMEM, GMEM, JMEM, RPMI, etc. For some types of cell culture, the use of serum-free media with or without protein is essential. Serum, serum proteins as well as a single amino acids can be replaced by recombinant proteins, peptides from protein hydrolysates and synthetic oligopeptides (Franek and Fussenegger, 2005).

Selular and Microenvironment Engineering

The usage of cellular and metabolic engineering aims to improve certain crucial cellular functions. the behavior of the cell towards the conditions of environmental stress, absence or expression of an inadequate level of endoplasmic reticulum and golgi enzymes necessary for co and post translational modification. Some examples are the usage of GS to select and optimize a system as to obviate the glutamine need in the culture medium; the overexpression of cytosolique pyruvate carboxylase in continuous cell lines to improve glycolysis and Krebs-cycle (Elias *et al.*, 2003); the overexpression of antisense of LDH-A cytoplasmic glycerol-3- phosphate dehydrogenase to increase oxidative phosphorylation and decrease cellular respiration and thus reduce the sensitivity to reactive oxygen species reducing cell apoptosis (Jeong *et al.*, 2004)

The tissue microenvironment is an important factor to regulate a normal cellular function. In other hand, cell culture limitation is the lack of natural structure and live architecture. In cell culture (in vitro condition), Not all cell types can adhere and grow well and capable to produce optimally natural signals. The microenviroment play critical role in the normal expression of cell phenotypes. The extra-cellular signal affects intracellular genes expression in turn the result of fundamental changes in the composition of the microenvironment. Inappropriate changes interactions cellular microenvironment may

produce abnormal cellular behavior, as seen in the development of the tumor; for example an ectopic implantation of embryonic cells progress become malignant tissue while the same cells located in the uterus has a normal embryo (Kim *et al.*, 2004).

Cell interaction and Three-Dimensional Culture

Signaling between cells through the adhesion molecules can provide the essential elements necessary to maintain normal cell physiology. Recent studies of functional integrins provide evidence that the structural stability, provided by the interaction of the Integrin-mediated cell-extra cellular matrix, is an important determinant of normal cells behavior while the expression of E-cadherin (cell-cell adhesion molecule) promote differentiation and inhibits the metastasis of severals cancer cell lines (Jones *et al.*, 1992). three dimensional cell growth can lead the cell differentiation and enhance the expression of E-cadherin and other cell adhesion molecules. But the fate of cells depends not only on the adhesions mechanism, but also the interaction of the adhesion molecules with soluble regulatory growth factors, such as hormones, cytokines and growth factors. For example, normal stroma could be protective in delaying or preventing the tumour formation. A normal stroma and its products can alter the adjacent cell (in the absence of malignant epithelial cells) and promote the phenotypic and genomic changes in different epithelial cells but an abnormal stroma can promote oncogenic activities including manipulation of metalloproteinases, inflammatory cell recruitment and changes the stromal signals (Ronnov-Jessen *et al.*, 1995)

In three-dimensinonal environment, the cell polarity increase thus stimulate the differentiation of normal epithelial cells, some matrix extra cellular increase their biological activities and narrowed Integrin usage compared to two-dimensional substrates, furthermore these matrix promotes better adhesion, migration and morphogenesis of cells

(Cukierman *et al.*, 2001). The overexpression of β1-Integrin and epidermal growth factor (EGRF) in malignant epithelial cells is evident of a cross-modulation between β1-Integrin and EGRF in human breast cancers cell culture (Wang *et al.*, 1998). The changes in intrinsic cell function greatly affects the response of a tissue model for an external agent (drugs, growth factor, hormone or other protein additive)

Scaffold

Recent developments in this field is the usage of synthetic biomaterials to create micro-environments mimic natural extracellular matrix. The specific goals is allowing stem cell differentiation and morphogenesis to the target tissue. The main requirements for scaffolds are biocompatibility and the ability to maintain or promote the growth of relevant cells and providing a good model of tissue growth in three-dimensiononal environment (Polak and Bishop, 2006). The decellularized tissue (allogeneic or xenogeneic) have frequently been used although it can be inoculated with primary cells (Kim et *al.*, 2004) . Collagen is the most scaffold commonly used but other extra cellular matrix proteins can also be used with the possibility additional of growth factors and other regulation subtances.

There are certain limitations associated with biological material used, including a lack of consistency and flexibility. The biological materials is divided into three groups: bioinert, resorbable, and bioactive. Bioinert is the raw material that almost completely inert when implanted (e.g. stainless steel). Absorbable materials are the materials that eventually dissolve after implanted. A variety of materials have been used, such as polymer (e.g. Polyglycolic, polylactic acid) is usually used for suture. bioactive materials is the ingredient that stimulates the body's biological response. Its can be classified as osteoconductive (e.g. synthetic hydroxyapatite ceramic) bind and stimulate of bone growth on the edge of the

graft and osteoproductive (e.g. bioactive glass) stimulate growth of new bone within material (Jones and Hench, 2003)

Cells can be harvested by using pre-fabricated Scaffolds. Cells adhere and migrate along intertwined scaffold fibers. When the cells divides, they fill interstices within the scaffold to form a three dimensional culture. Other type is pre-fabricated scaffolds composed by natural or synthetic polymer molecules that can be used as a three dimensional physical support matrix both in vitro culture and in vivo tissue regeneration. This scaffolding capable to recreate physical properties and structure environment of living tissue promoting extra celluar matrix molecule signaling pathways such as migration, proliferation and differentiation (Tan *et al.*, 2001).

A nanotopography controlling mechanical properties, and tissue engineering scaffold mechanical loading environment, should improve the regulation of stem cell fate in bioartificial systems. The adhesion molecules contribute to asymmetric stem cell division including β1-integrin, CD146, and E-cadherin, have begun to be identified in the environmental niche of the hair follicle, gut epithelium, and spermatogonial stem cells, respectively (Kanatsu and Shinohara, 2008; Oatley and Brinster, 2008). Cell-cell Interactions in the stem cell niche can be highly influenced by paracrine signaling (Kasper, 2008).

Recent Benefits of Cell Culture Technology

Cell culture contribute to produce biological materials such as vaccines, monoclonal or polyclonal antibodies, interferon, erythropoietin, hormones, clotting factors, immunoadhesin. Most of the biological material is a glycoprotein secreted by embryonic kidney cells such as human culture (HEK-293), Chinese hamster ovary (CHO) cells, and hybridoma cells (murine myeloma). In biopharmaceutical, cells culture used to investigate the drug activities and the design of new therapy strategies.

One of the new therapy is autologous cell therapy. Autologous cell therapy allows to take a small portion of the tissue and proliferated in vitro and than used in the body of the individual to the therapy. Most forms of therapy with this method is still in research or in clinical trials process. Research in, bone marrow mononuclear cell transplantation for acute radiation victims, autologous hematopoietic cells as gene transfer targets for the treatment of various blood disorders and autoimmune diseases, stroke ischemia, cardiac ischemia, leg ischemia cell-mediated immunotherapy after chemotherapy, and autologous cells for cancer therapy.

The cell culture can also be used to patient therapy in the form of cell transplantation, artificial organs and tissue engineering. Another development is a therapy based on gene therapy using a viral vector and development of artificial organs or tissue engineering based on the use of stem cell technology. The tissue repair is possible based on replacement tissue using stem cells. For this purpose, adult as well as embryonic stem cells human can also be used. Stem cells are defined as undifferentiated cells that can proliferate and the ability both to self renew and to differentiate to one or more types of specialized cells. The challenge now is to optimize the isolation and proliferation of cells, the differentiation of stem cells and to develope the scaffolding design and delivery systems that promote coordinated growth of three-dimensional tissues. Currently, four therapeutic approaches using stem cells have been identified : 1) the direct administration of adult stem cells, 2) regeneration mediated by stimulation of endogenous stem cells, 3) the introduction of differentiated stem cells and 4) tissue engineering. The majority of stem cell in clinic applications are based on the application of an adult or foetal stem cells. For example, intraoperative orthopedic procedures using adult stem cells have been developed by using adult osteogenic precursor cells, harvested from bone marrow, which is grown on a scaffold, they have an advantage over the adult cells of the placement of new extracellular matrix,

without remove the old matrix as in the adult bone autografts (Muschler and Midura, 2002). Embrionic Stem cell (ESC) may be induced to form osteoblast in vitro and in vivo, provide a source for bone tissue engineering. After implantation, the tissue will be able to survive and restore normal function and can integrate with the surrounding tissue (Bielby *et al.*, 2004),.

In the cardiovascular system, the delivery of autologous bone marrow stem cells is used for the treatment of myocardial infarction. ESC differentiated cardiomyocytes have been tested to repair the hearts of animal model cardiomyocytes regenerated and introduction of undifferentiated murine ESC contained in the liquid matrix into infarcted area increases cardiac contractility (Kofidis *et al.*, 2004). Human embrionic stem cell have been differentiated into endothelial cells isolated with antibodies to platelet endothelial-1 cell adhesion molecule (Levenberg *et al.*, 2002).

Future Prospect

Although cell culture media were developed but the ideal general purpose media has not yet been developed. Development and optimization of serum-free media to produce cell lines, the use of viral vectors for gene therapy, cell line and vector production are promizing. Future developments in the three-dimensional culture is a three-dimensional spheroids culture, This is a model heterologous multicellular complexes comprising complex cell-cell and cell-matrix interaction, culture organotypic composed of several types of cells, matrix and other environmental factors to simulate the overall environment in the culture of organs. Three dimensional spheroid culture is a valuable model to develop and test various types of drugs or treatment of degenerative diseases, cancer, gene therapy, influence immunocompetent cells to normal or abnormal cells differentiation and proliferation. The role of the stem cell niche also provide a potential therapeutic target for

regenerative medicine in the future. In order to complete the optimization of high performance, three dimensional culture should be combined with genomics and proteomics, so that it becomes a very powerful tool to analyze cell behavior under different culture conditions and develop gene therapy

Despite cell culture techniques showed a significant progress, but the final success of its utilization require a in vivo confirmations, so a multidisciplinary approach in the development of cell and tissue engineering involves many disciplines such as cell and molecular biology, biomedical engineering, medicine, material sciences and other related sciences.

References

Bielby RC., Boccaccini, AR., Buttery, LDK *et al.* 2004. In vitro differentiation and in vivo mineralization of osteogenic cells derived from human embryonic stem cells. Tissue Eng. **10:** 1518–1525.

Cukierman E, Pankov R, Stevens DR, Yamada KM., 2001. Taking cell-matrix adhesions to the third dimension. Science 294(5547): 1708–1712

Elias, CB, Carpentier E, Durocher Y, Bisson L, Wagner R, Kamen A., 2003. Improving glucose and glutamine metabolism in human HEK 293 and Trichoplusia ni insect cells engineered to express a cytosolic pyruvate carboxylase. Biotechnol Prog 19:90–97

Enders JF, Weller TH, Robbins FC (1949) Cultivation of Lansing strain of poliomyelitis virus in culture of various human embryonic tissues. Science 109:85–87

Franek F, Fussenegger M., 2005. Survival factor-like activity of small peptides in hybridoma and CHO cells cultures. Biotechnol Prog 21:96–98

Harrison RG., 1907. Cell Culture. Proc. Soc. Exp. Biol. Med, 4, 140.

Jeong DW, Kim TS, Cho IT, Kim IY., 2004. Modification of glycolysis affects cell sensitivity to apoptosis induced by oxidative stress and mediated by mitochondria. Bio- chem Biophys Res Commun 313:984–991

Jones JL, Critchley DR, Walker RA., 1992. Alteration of stromal protein and integrin expression in breast – a marker of pre- malignant change. J Pathol 167: 399–406,

Jones, JR and Hench, LL., 2003. Regeneration of trabecular bone using porous ceramics. Curr. Opin. Solid State Mat. Sci. **7:** 301–307.

Kanatsu-Shinohara, M., Takehashi, M., Takashima, S., Lee, J., Morimoto, H., Chuma, S., Raducanu, A., Nakatsuji, N., Fassler, R., and Shinohara, T., 2008. Homing of mouse spermatogonial stem cells to germline niche depends on beta1-integrin. Cell Stem Cell 3, 533–542.

Kasper, S. 2008. Exploring the origins of the normal prostate and prostate cancer stem cell. Stem Cell Rev. 4, 193–201.

Kim, JB., Stein R., O'Hare MJ., 2004. Three-dimensional in vitro tissue culture models of breast cancer. Breast Cancer Research and Treatment 85: 281-291.

Kofidis, T., De Bruin, JL., Hyot, G. Et al. 2004. Injectable bioartificial myocardial tissue for large-scale intramural cell transfer and functional recovery of injured heart muscle. J. Thorac. Cardiovasc. Surg. **128:** 571–578.

Levenberg S., Golub J S., Amit M., et al., 2002. Endothelial cells derived from human embryonic stem cells. Proc. Natl. Acad. Sci. USA **99:** 4391–4396.

Merten, OW. 2006. Introducing to Animal Cell Culture Technology-past, present and future. Cytotechnology 50:1-7

Montagnon BJ, Fanget B, Vincent-Falquet JC., 1984. Industrial-scale production of inactivated poliovirus vaccine prepared by culture of Vero cells on micro carrier. Rev Infect Dis 6(S2):210–213

Muschler, G and Midura, RJ., 2002. Connective tissue progenitors: practical con- cepts for clinical applications. Clin. Orthop. **395:** 66–80.

Nicolas AJ, Patet J, Vincent-Falquet JC, Branche R, Delaiti P, Montagnon B, Peyron L, Soulebot JP. 1978. Production of inactivated rabies vaccine for human use on WI38 diploid cells. Results of potency tests, stability of the vaccine in liquid and freeze-dried forms. Dev Biol Standard 40:17–24

Oatley, JM and Brinster, RL., 2008. Regulation of spermatogonial stem cell self-renewal in mammals. Annu. Rev. Cell Dev. Biol. 24, 263–286.

Polak, JM and Bishop, AE 2006. Stem Cells and Tissue Ewngineering: Past, Present and Future. Ann. N.Y. Acad. Sci. 1068: 352-366

Ronnov-Jessen L, Petersen OW, Koteliansky VE, Bissell MJ, 1995. The origin of the myofibroblasts in breast cancer. Recapitu- lation of tumor environment in culture unravels diversity and implicates converted fibroblasts and recruited smooth muscle cells. J Clin Invest 95: 859–873, 1995

Tan W, Krishnaraj R, Desai TA., 2001. Evaluation of nanostructured composite collagen–chitosan matrices for tissue engineering. Tissue Eng 7: 203

Wang F, Weaver VM, Petersen OW, Larabell CA, Dedhar S, Briand P, Lupu R, Bissell MJ., 1998. Reciprocal interactions between β1-integrin and epidermal growth factor receptor in three-dimensional basement membrane breast cultures: a different

perspective in epithelial biology. Proc Natl Acad Sci USA 95:14821–14826