REVIEW ARTICLE

Insect Cell Line Development, Maintenance and Susceptibility to Viral Infection: A Review

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ABSTRACT

In the last two decades, great attention has been directed to insect cell culture technology due to its useful role in biotechnology applications. For instance, they have been used to produce recombinant therapeutic proteins, vaccines, biopesticides as well as it is used in gene therapy. A few insect cell lines are available in the markets compared to the huge number of insect species. Establishing an insect cell line requires intensive effort and patience. Particularly, in the early stages of developing a cell line, normally, there will be many obstacles, such as microbial contamination and cell adaptation to the new environment. Finding a good method for tissue disinfection and providing suitable growth conditions such as insect cell culture media and temperature are considered one of the critical keys to developing successful cell lines. This review discusses the requirements to develop insect cell lines, maintenance, and viral infection.

Keywords: Insect cell line, Cell line development, Insect cell culture media, Virus infection.

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INTRODUCTION

Selecting Insect Tissues for Developing Cell Lines

The currently available insect cell lines have been developed from various mature and immature tissues. Various tissues, for instance, embryos, midgets, fat bodies, hemocytes, and ovaries, have been used to establish cell lines that will be used later on for various applications.[1-4] However, the type of insect tissue may play an important role in developing a successful continuous cell line.[5]

The probability of establishing new successful cell lines is high using immature tissues comparing to mature tissues.[6] Therefore, several researchers have used embryonic tissues to establish new insect cell lines.[4,7] Nevertheless, at the same time, the cells that are established from mature tissues are characterized by similarity.[6,8] However, immature and mature tissues have been used in the initiation of insect cell cultures.

Tissue Disinfection Methods

The first step in the process of developing an insect cell line is to get rid of microbial contaminants, which consider big challenges. Therefore, researchers used several different procedures for disinfecting insect tissues. Various disinfection solutions have also been used in this regard. The most commonly used disinfection solutions are ethanol and sodium hypochlorite.[4,6]

After disinfection, the disinfected tissues are cut into small pieces and transferred into one suitable medium have antibiotics.[6,9] However, different antibiotic types and numbers have been used to avoid microbial contamination; some researchers used only one antibiotic while others used two and more different types. The following antibiotics: gentamycin, penicillin and streptomycin, are used widely in cell culture.[3,9] It is recommended to use antibiotics during the initiation of primary cell culture and after that for only a short time when maintaining the cell culture.

The presence of antibiotics in the insect cell culture medium does not mean that the antibiotics kill all the microorganisms 100% but it could reduce their population and be invisible. When the bacteria be resistant with time, then it will be visible in the culture. Consequently, the researchers will lose time, effort and money Therefore it is quite easy to observe any microbial contamination in early stages if no antibiotics present in the media.[10]

It is more worth if it can establishing cell lines without using antibiotics. Researchers have succeeded in developing cell lines from insect embryonic tissues without using any antibiotics; they have focused only on using disinfection solutions.[4]

Insect Cell Culture Media

One of the keys to developing successful cell lines is to provide the essential nutrients that insect cells needed to grow and

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divide. Several compounds are essential to insect cell growth such as carbohydrates, amino acids, growth factors, hormones, lipids, vitamins and inorganic salts. Carbohydrates, which are considered a source of energy and carbon, are important to the insect cell culture and amino acids are important sources of nitrogen. From 20 amino acids, only 15 amino acids are essential for insect cells.[11,12]

In 1956, Wyatt and his group were the first researchers who attempt to prepare a medium for cultivating insect cells. The medium composition was to mimic the hemolymph of Bombyx mori. It contained beside the heat-treated hemolymph: sugars, organic acids, inorganic acids and amino acids.[13,14] Later on, other researchers modified the medium to make it suitable for culturing cells established from insects belonging to Lepidoptera.[15] This medium, called Grace's medium, it was turned to be more acid (6.2–6.9) and the pH can be adjusted with sodium phosphate. Therefore no need for Co_2 to maintain the pH of the media like mammalian cells which require Co_2 to control the pH of the media. Since a long time, Grace's medium has been commercially produced and available in the market.[16]

It is well known that there are a huge number of insect species living in different environments. Therefore, the nutrient requirements are not the same for all insect species. Therefore, several different media have been manufactured to cover the necessary demand for each cell line.[17] Some modifications have been made to Grace's medium the modified medium contained fetal bovine serum (FBS), lactalbumin and yeast late.[14,18] FBS is not cheap, making the insect cell culture media very expensive therefore there was an urgent need to develop low-cost media, especially after increasing the industrial application of insect cell lines.[19] In the following Table 1 are some examples of available media:

Initiation of Primary Cell Culture

Several cell lines have been established from different insect species mostly belong to the order of Lepidoptera.[11,20] The primary cell culture refers to the culture made by cutting the animal tissues to tiny pieces and incubating them under suitable cell culture conditions in the essential medium until the first subculture.[21] The small size of the insects makes starting the primary culture arduous.[6] Establishing the primary culture begins with the acquisition of the sample. Then, the tissue must be isolated and dissected and/or desegregated. After that, the culture is seeded into the culture vessel and incubated at 26ºC.[21]

Table 1: Insect cell culture media

Name of medium	*Company*
Grace's medium	
IPL-41	
ExpiSf CD Medium	Life Technologies
Express Five SFM	
Sf-900 III SFM	
TC-100	
EX-CELL™ 405	Sigma-Aldrich
TNM-FH	
EX-CELL 420	
Insect-XPRESS™ Protein-free	Lonza, USA

Cell Subculture and Maintenance

After initiation of cell culture, the culture medium has to be replaced at least one time a week to provide the culture with fresh nutrients and remove toxins. In the early stages of the primary cell culture, a net of structures similar to nerves as well as big vesicles could be found in the cultures.[4,22,23] The primary culture can survive for several months and can be subcultured thereafter or die. However, these primary cultures have to be maintained by replacing half of the old medium with fresh medium each week to reduce the toxic metabolites and supply enough nutrients for the cells. The cells have to be spilt when they cover most of the cell culture vessel surface and reach a confluent state. The first subculture is considered an important transition from a primary culture to a cell line. However, the first successful subculture can take weeks, months, or even more than a year, depending on the tissues' insect species and origin.[3,4] The first subculture can take from a few weeks to more than a year.[4,24] Adherent and suspension cells can be produced from the explants.4 The cells have to be split before reaching confluence to provide them with enough space and fresh nutrients.

Researchers reported that earlier passages after the primary culture of the cells were grown slowly and the intervals between subcultures take a long time. However, with advancing subcultures, the cells able to grow faster and the intervals between subcultures become shorter after a certain time the cells will be passaged at regular intervals.[11]

Insect Cell Line

The term cell line can be referred to as the cell culture after the first successful subculture and increasing cell density again. The cells could stop dividing, lose their viability, and die after a certain number of passages, and these cell lines are called finite cell lines. Simultaneously, the cells that can be subcultured forever are known infinite or continuous cell lines.[25]

The normal cells can be divided for a definite number of times and then they die in the process called senescence. Some cell lines can grow continuously, which could be due to genetic variation from deletion or mutation of genes responsible for cell cycle progression arrest. The transition of primary cell culture to a continuous cell line is called in vitro transformation.[25] The evolution of a cell line is demonstrated in Figure 1. Continuous cell lines have been successfully developed from insect tissues since 1962. The first successful insect cell line was established from *Antherea pernei* moth ovaries by Grace in 1962. Since then, many insect cell lines have been established from different insect tissues.

According to the literature, the doubling time for most of the common insect cell lines, which are established from Lepidoptera, is in the range between 18 to 30 hours.[26]

More than 500 cell lines have been established from various insect orders such as Lepidoptera, Diptera, Hymenoptera, Heteroptera, Dictyoptera, Orthoptera and Coleoptera.[27] The

Table 2: Examples of biopharmaceutical products produced in insect cells

Product	*Application*	*Company*	*Reference*
CERVARIX®	Vaccine	GSK	[40]
CircoFLEX®	Vaccine	Boehringer Ingelheim	
Porcilis® PCV	Vaccine	Intervet-Schering-Plough	
Provenge®	Prostate cancer immunotherapy	Dendreon	
Glybera®	gene therapy	uniQure	[41]
Flublok®	Seasonal influenza vaccine	ProteinsSciences corporatio	

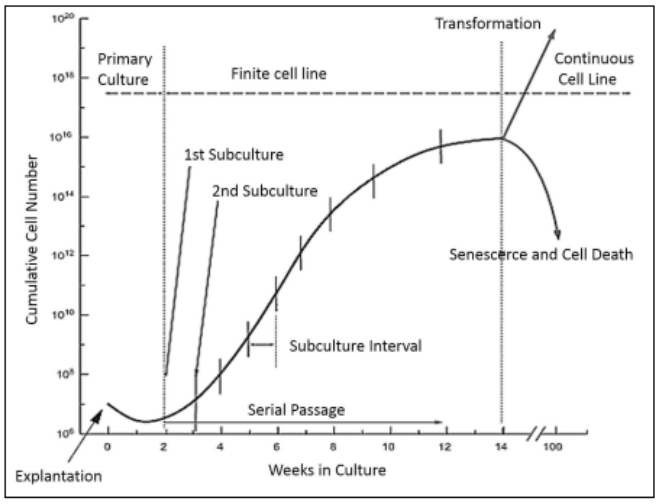

Figure 1: Cell line development.[21]

cell lines: Sf21, Sf9 and Tni are the most used in therapeutic protein manufacturing.[28]

Cryopreservation of Insect Cell Lines

Insect cells can be stored for short-term at −80 °C and for long-term storage, the cells have to be store in liquid nitrogen.[29] The cells have to be at the logarithmic phase when they are harvested to generate a cell bank and suspended in a freezing medium, which contains an insect medium with 10% DMSO. It is highly recommended to store some of the cells after certain cell subcultures even in early passages while establishing a cell line. Storage of cell lines is important to prohibit losing valuable cell lines and prevent microbial contamination, which could happen during the *maintenance* of cell lines.

Cell Line Susceptibility to Viral Infection

Insects like other organisms can be infected by various pathogens including viruses. More than 1100 various viruses are available in the environment that can infect insects belong to different insect families.[30] The insect viruses can have RNA or DNA genome according to the literature there are 6 DNA and 12 RNA viral families in total, around 18 viral families that can infect insects.[31] Baculoviridae is one of the most common viral families that infect insects and the viruses belong to this family called Baculoviruses. The life cycle of baculovirus starts when insect larvae eat food contaminated with viral occlusion bodies (OBs) the alkaline environment of the larvae stomach dissolve the OBs and release occlusion derived virus (ODV) which infect epithelial cells. The secondary infection is caused by a budded virus (BV) produce by infected cells.[32]

Baculoviruses are divided into Granulovirus (GV) and Nucleopolyhedro virus (NPV) depending on the protein matrix, which the occlusion-derived virus is embedded inside. Because baculoviruses are insect-specific therefore the virus' s name is taken from the insect host.[33]

After establishing insect cell lines, they are infected with baculovirus to test these cell lines' susceptibility to a certain type of baculoviruses. As wide applications of insect cells in biotechnology, this requires that the insect cells have to be susceptible to viral infection in order to be able to produce the virus particles as biopesticide or recombinant protein production.[34,35]

Application of Insect Cell Lines

The insect cells have been proved high efficiency in producing various active recombinant proteins for both human and veterinary use.[36,37] The insect cells can produce human complex proteins with similar to human glycosylation patterns, which cannot be produced by bacteria.[38,39] Table 2 shows some of the commercial therapeutic proteins produced in insect cells and available in the market.

REFERENCES

1. Baines D, Brownwright A, Schwartz J. Establishment of primary and continuous cultures of epithelial cells from larval lepidopteran midguts. Journal of Insect Physiology. 1994;40(4): 347-357.
2. Goodman C, El Sayed G, Mcintosh A, Grasela J, Stiles B. Establishment and characterization of insect cell lines from 10 lepidopteran species. In Vitro Cellular & Developmental Biology - Animal. 2001;37(6):367-373.
3. Belloncik S, Petcharawan O, Couillard M, Charpentier G, Larue B, Guardado H et al. Development and characterization of a continuous cell line, AFKM-On-H, from hemocytes of the European corn borer Ostrinia nubilalis (Hübner) (Lepidoptera, Pyralidae). In Vitro Cellular & Developmental Biology - Animal. 2007;43(7):245-254.
4. Ahmed I, Huebner H, Buchholz R. Establishment and characterization of three new embryonic Spodoptera littoralis cell lines and testing their susceptibility to SpliMNPV. JSM Biotechnology and Biomedical Engineering. 2014;2:1031.
5. Lynn D. Development of insect cell lines: Virus susceptibility and applicability to prawn cell culture. Methods in Cell Science. 1999;21(4):173-181.
6. Lynn D. Development and characterization of insect cell lines. Cytotechnology. 1996;20(1-3):3-11.

7. Goblirsch M, Spivak M, Kurtti T. A cell line resource derived from honey bee (Apis mellifera) embryonic tissues. PLoS ONE. 2013;8(7):e69831.
8. Baines D. New approaches to insect tissue culture. Cytotechnology. 1996;20:13-22.
9. Harrison R, Lynn D. New cell lines derived from the black cutworm, Agrotis ipsilon, that support replication of the A. ipsilon multiple nucleopolyhedrovirus and several group I nucleopolyhedroviruses. Journal of Invertebrate Pathology. 2008;99(1):28-34.
10. Ahmed I. Establishment and characterization of three new embryonic Spodoptera littoralis cell lines and testing their susceptibility to SpliMNPV. Doctoral Thesis. FriedrichAlexander-Universität Erlangen-Nürnberg (FAU), Technische Fakultät. 2015.
11. Mitsuhashi J. Invertebrate tissue culture methods. Springer Lab Manual. 2002:791-812.
12. Claus J, Gioria V, Micheloud G, Visnovsky G. Production of insecticidal baculoviruses in insect cell cultures: potential and limitations. In: Insecticides-Basic and other Applications. Soloneski S, Larramendy M (eds.), InTech. 2012;127-152.
13. Wyatt G, Loughheed T, Wyatt S. The chemistry of insect hemolymph. Journal of General Physiology. 1956;39(6):853-868.
14. Schlaeger E. Medium design for insect cell culture. Cytotechnology. 1996;20(1-3):57-70.
15. Grace T. Establishment of four strains of cells from insect tissues grown in vitro. Nature. 1962;195(4843):788-789.
16. Drugmand J. Characterization of insect cell lines is required for appropriate industrial process. Case study of High five cells for recombinant protein production. Thesis. 2007.
17. Stork N. How many species of insects and other terrestrial arthropods are there on earth? Annual Review of Entomology. 2018;63(1):31-45.
18. Hink W. Established insect cell line from the cabbage looper, Trichoplusia ni. Nature. 1970;226:466-467.
19. Agathos S. Development of serum-free media for lepidopteran insect cell lines. Methods in Molecular Biology. 2007;388:155-186.
20. Lynn D. Available lepidopteran insect cell lines. Methods in Molecular Biology. 2007;117-137.
21. Freshney R. Culture of animal cells: A manual of basic technique. Wiley-Blackwell. 2005.
22. Takahashi M, Mitsuhashi J, Ohtaki T. Establishment of a cell line from embryonic tissues of the fleshfly, Sarcophaga peregrine (Insecta: Diptera). Development, Growth and Differentiation. 1980;22:11-19.
23. Rey G, Ferro C, Bello F. Establishment and characterization of a new continuous cell line Lutzomyia longipalpis (Diptera: Psychodidae) and its susceptibility to infections with Arboviruses and Leishmania chagasi. Memórias de Instituto Oswaldo Cruz. 2000;95:103-110.
24. Dübendorfer A, Liebig B. Cell differentiation in vitro and establishment of permanent ecdysone-responsive cell lines from embryonic tissue of the Colorado potato beetle, Leptinotarsa decemlineata. Journal of Insect Physiology. 1992;38:397-407.
25. Freshney R. Culture of animal cells: A manual of basic technique and specialized applications. Wiley-Blackwell. 2010.
26. Thermo fisher Scientific Catalog. Growth and maintenance of insect cell lines. USER GUIDE http://tools.thermofisher.com/content/sfs/manuals/Insect_Cell_Lines_UG.pdf. 2017.
27. Lynn D. Novel techniques to establish new insect cell lines. In Vitro Cellular and Developmental Biology – Animal. 2001;37(6):319-321.
28. Zitzmann J, Sprick G, Weidner T, Schreiber C, Czermak P. Process optimization for recombinant protein expression in insect cells. New insights into cell culture technology. 2017.
29. Vyletova L, Rennalls L, Wood K, Good V. Longterm, large scale cryopreservation of insect cells at 28°C. Cytotechnology. 2016;68:303-311.
30. Grzywacz D. Basic and applied research: Baculovirus. Chapter 3. Microbial Control of Insect and Mite Pests: From Theory to Practice. Elsevier Inc. 2017.
31. Hu Z, Chen X, Sun X. Molecular biology of insect viruses. Advances in Microbial Control of Insect Pests. Springer Science+Business Media, LLC. 2003;83-107.
32. Rohrmann G. Baculovirus Molecular Biology. 4th edition. Bethesda (MD): National Center for Biotechnology Information (US) [https://www.ncbi.nlm.nih.gov/books/NBK543458/]. 2019.
33. Goodman D, Ollikainen N, Sholley C. Baculovirus phylogeny based on genome rearrangements. In: Tesler G., Durand D. (eds) Comparative Genomics. RECOMB-CG. Lecture Notes in Computer Science, vol 4751. Springer, Berlin, Heidelberg. 2007.
34. Lynn D. Comparative susceptibilities of insect cell lines to infection by the occlusion-body derived phenotype of baculoviruses. Journal of Invertebrate Pathology. 2003; 83: 215-222.
35. Arif B, Pavlik L. Insect cell culture: Virus replication and applications in biotechnology. Journal of Invertebrate Pathology. 2012; Suppl: S138-141.
36. Madhan S, Prabakaran M, Kwang J. Baculovirus as vaccine vectors. Current Gene Therapy. 2010;10(3):201-213.
37. VanOers M, King L. The application of baculoviruses in human and veterinary medicine: An overview. Journal of Invertebrate Pathology. 2011;107:S1–S2.
38. Cox M. Recombinant protein vaccines produced in insect cells. Vaccine. 2012;30:1759-1766.
39. Kollewe C, Vilcinskas A. Production of recombinant proteins in insect cells. American Journal of Biochemistry and Biotechnology. 2013;9(3):255-271.
40. Druzinec D, Salzig D, Brix A, Kraume M, Vilcinskas A, Kollewe C, Czermak P. Optimization of insect cell based protein production processes -online monitoring, expression systems, scale up. In A. Vilcinskas (Ed.), Advances in Biochemical Engineering/Biotechnology: Yellow Biotechnology II: Insect Biotechnology in Plant Protection and Industry. 2013;136:65-100.
41. Palomares L, Realpe M, Ramirez O. Chapter 15: An overview of cell culture engineering for the insect cell baculovirus expression vector system (BEVS). Springer International Publishing Switzerland. M. Al-Rubeai (ed.), Animal Cell Culture, Cell Engineering. 2015;9.