Egyptian Journal of Biological Pest Control

REVIEW ARTICLE

Open Access

Insect cell culture vis-à-vis insect pest control

Jabez Raju Battu[1*], Karthik Somala[2*], Yashaswini Gummudala[3], Shankara Sai Reddy Morthala[2], Karthik Ramappa[4], Anil Gadde[5] and Nitika Negi[4]

Abstract

Background Insect pests are a major cause for losses in agricultural systems, and it is reported that they alone cause up to 45% loss in annual food production. Alleviating the destructive upheaval caused by these six-legged mortals in the agroecosystems falls within the expansive docket of the scientific coterie. Hence, insects are a subject to many experiments in the laboratories and on fields to understand and evaluate their interactions with their biology, physiology, and behavior so as to develop effective managerial strategies against them. Conventional methods include rearing the insects in the laboratory for experimenting on them, which is a very tiring as well as time-consuming process. How convenient it would be, if there is a way to conduct experiments which are directed specifically toward the tissues of insects, particularly cells.

Main body The present review presents the immense potential of insect cell cultures in screening the toxicity and mode of action of novel insecticides, physiological studies apart from their ability to produce recombinant proteins through baculovirus expression vector system (BEVS) which includes a broad range of molecules ranging from the antibiotics to the vaccines. Also, we bring together the concept of culturing insect cells in vitro and how revolutionary they could be in changing the future of research in burgeoning strategies to tackle the menace of insect pests in agricultural production systems.

Conclusion A deeper grasp of biology and physiological processes will enable us to create techniques that will improve our arsenal in the fight against food crop insect pests. The advancement in culturing insect cells and their potential in entomological research aimed at developing pest control strategies and also for manufacturing vaccines.

Keywords Insect cell culture, Insect pest management, BEVS, Vaccine

Background

Science has always been a propulsive force in the establishment and development of modern human communities breaking through the obstacles and equipping us for the future. With the advent of microscope in the sixteenth century, scientists began to study living organisms under the limelight of the microscope. Discovery of cells by the British scientist Robert Hooke in 1665 revolutionized the scientific work carried out across the globe. Cells of living organisms were isolated, and efforts were made to grow them in the laboratories in vitro under controlled conditions. In the late nineteenth century, Wilhelm Roux was the first person to culture living cells from the neural plate of chick embryo in a saline buffer. Then again

*Correspondence:
Jabez Raju Battu
yebbetzyisrael@gmail.com
Karthik Somala
karthiksomala13@gmail.com
[1] Department of Biological Sciences, Clemson University, Clemson, USA
[2] Department of Entomology, Dr. Rajendra Prasad Central Agricultural University, Pusa, India
[3] Department of Entomology, Professor Jayashankar Telangana State Agricultural University, Hyderabad, India
[4] Department of Entomology, Chaudhary Sarwan Kumar Himachal Pradesh Krishi Vishvavidyalaya, Palampur, India
[5] Department of Entomology, Acharya N.G Ranga Agricultural University, Bapatla, India

in 1907, an American Scientist Ross Harrison cultured cells from embryonic tissues of frogs in their lymph in his experiments, which later would be popularly known as 'the hanging drop technique' (Jedrzejczak-Silicka 2017). The first attempt at culturing insect cells in vitro was made by Goldschmidt, who in 1915 cultured follicle cells from the gonads of male pupae of *Hyalophora cecropia* (Saturniidae: Lepidoptera) in the hemolymph of the same insect (Goldshmidt and Wilhelm 1915). In 1935, William Trager was the first person to successfully culture baculovirus in primary ovarian cell cultures of *Bombyx mori* larvae (Bombycidae: Lepidoptera) and published his observations (Trager 1935). Following Trager's work, Silver Wyatt tried to improve the media prepared by Trager and was successful in her attempts. She used inorganic salts, sugars, organic acids and amino acids altogether 34 ingredients, unlike Trager who used 6 ingredients (Wyatt 1956). Shangyin Gaw first reported the successful establishment of insect cell line in 1959 (Gaw et al. 1959). Thomas Grace improved the medium prepared by Wyatt by adding vitamins, adjusting the ionic ratios of Na/K and Ca/Mg, changed the osmotic pressure and pH. Using this medium, he was succeeded in establishing the cell lines from ovarian tissues of *B. mori*, *Antheraea eucalypti* and larval tissues of *Aedes aegypti* (Culicidae: Diptera) (Grace 1966, 1967). The medium prepared by him is still widely used today for culturing insect cells (Granados et al. 2007). Following the development of Grace's medium, Imogene Schneider in 1964 prepared his media for culturing antennal disk cells (Schneider 1964). Subsequently, several researchers attempted to modify existing media based on their requirements (Ghosh et al. 2020).

With the availability of suitable media for culturing insect cells, insect cell culturing progressed just from maintaining the cell lines to developing recombinant proteins and vaccines from the cultured insect cells. Hitherto, cell cultures of insects were used for studying cell biology and physiology, understanding metabolic pathways in insects as well as other living organisms, studying the viral infections and their transmission by insect vectors, toxicity assays, etc., besides this, they are also used for the production of recombinant proteins (Ghosh et al. 2020). Insects are infected with baculoviruses naturally, and scientists started using these baculoviruses in insect cell cultures to study their infection in insects and utilized them to express heterologous recombinant proteins in insect cell cultures. Ever since then, the baculovirus expression system (BEVS) was used to produce recombinant proteins in insect cells. This BEVS is now used for manufacturing vaccines right from the World's first HIV candidate vaccine and other vaccines in development using cultured cells (High five) from *Trichoplusia ni* (Noctuidae: Lepidoptera) (Puente-Massaguer et al. 2021) to the vaccine for Covid-19 disease-producing SARS-CoV-2 virus and its variants like Omicron as well from the cultured cells (Sf9 cells) of *Spodoptera frugiperda* (Smith) (Noctuidae: Lepidoptera) (van Oosten et al. 2021). This review aims to summarize the advancements in culturing insect cells and their potential in entomological research for developing pest control strategies.

Main text

Cell culture is the process of isolating cells from animal tissues and growing them successfully in an artificial culture media (Arunkarthick et al. 2017). The two main techniques for developing cells in culture are anchor dependent on an artificial substrate (monolayer culture) and free-floating in the culture media (suspension culture).

Types of insect cell cultures

Monolayer cultures

Monolayer or adherent cell cultures are cell lines that are anchor dependent and cultivated while attached to a substrate (Arunkarthick et al. 2017). These are suitable for most cell types, including primary cultures, and allow for easy visual inspection under an inverted microscope. Enzymatic or mechanical dissociation is used to separate cells. Surface area limits growth, which limits product yields and necessitates tissue-culture treated vessels. These cultures are utilized for cytology, continuous product harvesting, and many other research applications.

Suspension cultures

Suspension culture is an anchorage-independent culture where multiplication of small aggregates of cells or single cells takes place suspended in a liquid medium that is agitated (Willems and Jorissen 2004). Appropriate for cells acclimated to suspension culture as well as a few other non-adhesive cell types (hematopoietic cells). Passage is easier, but daily cell counts and viability determination are required to track growth patterns; culture can be diluted to accelerate growth. No enzymatic or mechanical dissociation is required. The concentration of cells in the medium limits their growth, allowing for efficient scale-up. It can be maintained in non-tissue-culture-treated culture vessels, but enough gas exchange requires agitation (shaking or stirring). These cultures are utilized for bulk protein production, batch harvesting, and other research applications.

Insect cell culturing media

Insect cell culturing is being used commercially for its expediency, and in fact it has become an important component of modern biotechnology. Therefore, improved media are needed that strongly support the cell growth.

Table 1 Different types of insect cell culture media

Media	Formulation	References
Conventional media		
Wyatt's medium	Medium consisted of high concentrations of organic acids, amino acids, inorganic salts and sugars supplemented with heat-treated hemolymph	Wyatt et al. (1956)
Grace medium	Medium consisted of 21 amino acids, four organic acids (Krebs cycle intermediates), ten vitamins, two antibiotics, six salts, three sugars, and insect plasma	Grace (1962)
MM insect culture medium	Medium consisted of only 6 salts, yeastolate, fetal bovine serum (FBS), lactalbumin hydrolysate, glucose, and antibiotics	Mitsuhashi (1964)
Hink's TNM-MH medium	Medium consisted of lactalbumin, yeastolate as source of vitamins (B-complex), and hemolymph with heat-inactivated fetal bovine serum	Hink (1970)
BML-TC/10	Medium consisted of glucose (only hexose sugar), fetal bovine serum and tryptose extract	Schlaeger (1996)
IPL-41 medium	Medium consisted of increased concentration of amino acids, vitamins and protein hydrolysates (lactalbumin, tryptose phosphate broth, yeastolate)	Goodwin (1975)
Serum media		
ISFM medium	Prepared based on IPL-41, ultrafiltered yeastolate ($4g1^{-1}$) and a complex lipid emulsion were added	Inlow et al. (1989)
Ex-Cell-400 medium	It's a semi-defined medium with a protein concentration of 15 $mg/m1^{-1}$ or less	Belisle et al. (1992)
ExCeU 401 medium	It's a protein-free medium that allows for higher cell density and higher yields of expressed recombinant proteins	Schlaeger (1996)
Sf 900 medium	It's a medium with low protein content	Weiss et al. (1992)

Different types of insect cell culturing media are enlisted in Table 1.

Conventional media

In order for the insect cells to flourish in vitro, they require an environment that promotes their proliferation outside of their original tissue. Consequently, early insect cell cultures were maintained in a basic mixture of vitamins, amino acids, carbohydrates, and salts supplemented with a poorly defined biological fluid, such as serum or hemolymph (invertebrates' circulatory fluid), known as conventional media (Schlaeger 1996). Moreover, for the growth of insect cell lines, serum rapidly became the favored supplement (Hink 1970).

Serum-free insect cell culture media

By replacing the serum with appropriate nutritional and hormonal compositions, serum-free medium (SFM) avoids the problems associated with utilizing animal sera. Many primary cultures and cell lines, including recombinant protein generating lines, numerous hybridoma cell lines, the insect lines Sf9 and Sf21 of *Spodoptera frugiperda* (Noctuidae: Lepidoptera), and cell lines that act as viral hosts (VERO, MDCK, MDBK), and others, have serum-free medium formulations (Li et al. 2021). One of the most significant advantages of employing serum-free media is the flexibility to make the medium to specific cell types by selecting the right mix of growth factors.

Serum has a variety of well-known drawbacks, including high cost, mycoplasma or contamination risk, low batch-to-batch repeatability, poorly defined composition, and challenges in downstream processing. These issues led to the creation of serum-free insect cell culture media, which has shown to be extremely useful in large-scale production. The low serum concentration has a sufficient growth-promoting effect, lowers total costs, protects cells from stress to some extent, and protects recombinant proteins from proteolytic attack. Indeed, several media for insect cell culture, such as Ex-Cell 401, Ex-Cell 405, Ex-Cell 420, Express Five, and SF900-II, are now commercially available and widely utilized (Rodas et al. 2005). Meanwhile, biochemical companies' intervention in insect cell culture has led to an increase in the number of commercial serum-free media. Table 2 lists the most serum-free (often protein-free) insect cell culture media currently available (Chan and Reid 2016).

Establishment of insect cell cultures

Insect cell cultures were established and maintained for use in a variety of scientific domains including insect pathology, toxicology, pesticide screening, and activity assay (Monti et al. 2014). To set up and maintain a cell culture, a small cell laboratory equipped with essential instruments is required. These instruments include a laminar flow hood for cell management and sterile reagents, an inverted phase-contrast microscope with 10× (or 20×) and 40× phase-contrast objectives, a mechanical pipetting device to dispense reagents, medium, and cells, a refrigerated incubator to maintain cells at 24–28 °C, sterilizers, autoclaves, UV air purifier are established.

Table 2 Serum-free media used for insect cell culture

Manufacturer	Medium	Insect cell lines
Allele Biotech	SFICM (Sapphire™)	Sf9, Sf21, Tn5
Applichem	AC Insect	Sf9, Tn5
BD Biosciences	Max-XP (BD BaculoGold™)	Sf9, Sf21
Biochrom AG	Insectomed SF Express	Sf9, Sf21, Tn5
Biological Industries	BIOINSECT-1	Sf9, Tn5
Corning	Insectagro Sf9™	Sf9, Sf21
Cosmo Bio	COSMEDIUM 009	Sf9
Expression Systems	ESF 921	Sf9, Sf21, Tn5
Expression Systems	ESF AF	Sf9, Sf21, Tn5
Irvine Scientific	IS BAC™	Sf9, Sf21, Tn5
Kohjin Bio	KBM710	Sf9
Life Technologies	Sf-900™ II	Sf9, Sf21, Ld, Tn368,
Life Technologies	Sf-900™ III	Sf9, Sf21
Life Technologies	Express Five ®	Tn5
Lonza	Insect-XPRESS™	Sf9, Sf21
Merck Millipore	Tri-Ex™	Sf9
MP Biomedicals	SFPFIM	Sf9, Tn5
Oxford Expression Tech	baculoGROW™	Sf9, Sf21, Tn5
Sigma-Aldrich	EX-CELL™ 420	Sf9, Sf21
Sigma-Aldrich	EX-CELL™ 405	Tn5
Sigma-Aldrich	TiterHigh™ Sf	Sf9, Sf21
Thermo Scientific	SFM4-Insect™ (HyClone ®)	Sf9, Sf21, Tn5
Thermo Scientific	SFX-Insect™ (HyClone ®)	Sf9, Sf21, Tn5
GENTAUR	MED-10002	Sf9, Sf21, Tn368, Tn5
GIBCO™ Invitrogen Corporation	Express Five™	Tn5
Hyclone	HyQ CCM3™	Sf9
MERCK Biosciences	BacVector™	Sf9

Selection of the proper medium is mandatory to maintain an insect cell culture media which should contain carbohydrates, amino acids, and salts at concentrations adapted to insect cell metabolism (Lynn 2007). The media used by insects is typically more acidic (pH: 6.2 to 6.9) and is buffered with sodium phosphate; osmotic pressure is also higher than mammalian cell culturing media. To develop new cell culture, a shotgun technique is used, in which every commercially available medium is explored (Lynn 2002). Commercial media like Ex-Cell 405 and SF900 for lepidoptera and Schneider's *Drosophila* medium for Diptera are being used.

Maintenance of insect cell cultures

Most primary cell cultures do not last longer than two months; yet, this short period is sufficient for a variety of studies and research, such as viral propagation in cultured cells and the study of immunocytes in mediating immune responses to various immunological stimuli (Smagghe et al. 2009). The morphology of the cells (form and appearance) must be examined using an inverted microscope every 2–3 days, and the date, name of the culture, and kind, amount, and particular source of the culture medium used.

Furthermore, evaluating the cells by eye with a microscope each time they are handled will help to spot any signs of contamination early on and contain it before it spreads to other cultures across the laboratory, confirming their healthy state. Granularity around the nucleus, cell separation from the substrate, and cytoplasmic vacuolation represent contamination of the culture, cell line senescence, or the presence of hazardous compounds in the medium, or the necessity for a medium change. As a result, contemporary laminar flow hoods and good aseptic procedures are enough to obviate the requirement for various antibiotics and antimycotic reagents in the stock culture maintenance.

Subculture of the cells

Subculturing, also known as passaging, entails the removal of spent media, adding new medium, and

Table 3 Insect cell lines used in entomological research

Cell line	Origin	Developed from	Medium	References
IPLB-LdEp	Lepidoptera	*Lymantria dispar* (Erebidae: Lepidoptera) embryos	Ex-Cell 400	McKelvey et al. (1996)
CP-169	Lepidoptera	*Cydia pomonella* (Tortricidae: Lepidoptera) embryos	TC199-MK	Hink and Ellis (1971)
ECIRL-PX2-HNU3	Lepidoptera	*Plutella xylostella* (Plutellidae: Lepidoptera) pupae	TC 199-M K	Carlo et al. (1983)
MRRL-CH	Lepidoptera	*Manduca sexta* (Sphingidae: Lepidoptera) embryo	Grace medium	Marks (1980)
IPLB-LdFB	Lepidoptera	*Lymantria dispar* (Erebidae: Lepidoptera) larval fat bodies	Ex-Cell 400	Lynn et al. (1988)
IPLB-TN-R2	Lepidoptera	*Trichoplusia ni* (Noctuidae: Lepidoptera) embryos	TNM-FH	Rochford et al. (1984)
FPMI-Cf-70	Lepidoptera	*Choristoneura fumiferana* (Tortricidae: Lepidoptera) pupal ovaries	–	Palli and Retnakara (1999)
IPLB-SF-21	Lepidoptera	*Spodoptera frugiperda* (Noctuidae: Lepidoptera) pupa ovaries	TNM-FH	Vaughn et al. (1977)
ECIRL-HA-AM1	Lepidoptera	*Helicoverpa armigera* (Noctuidae: Lepidoptera) pupal ovaries	TC199-MK	McIntosh et al. (1983)
IAL-PID2	Lepidoptera	*Plodia interpunctella* (Pyralidae: Lepidoptera) imaginal wing disks	TNM-FH	Lynn and Oberlander (1983)
IPLB-HVT1	Lepidoptera	*Heliothis virescens* (Noctuidae: Lepidoptera) testes	TNM-FH	Lynn et al. (1988)
ECIRL-HS-AM1	Lepidoptera	*Heliothis subflexa* (Noctuidae: Lepidoptera) pupal ovaries	TC199-MK	McIntosh (1991)
IPLB-DU 182E	Coleoptera	*Diabrotica undecimpunctata* (Chrysomelidae: Coleoptera) embryos	IPL-52B	Lynn and Stoppleworth (1984)
DSIR-HA-1179	Coleoptera	*Heteronychus arator* (Scarabaeidae: Coleoptera) embryos	TC 1 99-M K	Crawford (1982)
ERL-AG-1	Coleoptera	*Anthonomus grandis* (Curculionidae: Coleoptera) eggs	Ex-Cell 400	Stiles et al. (1992)
LD	Coleoptera	*Leptinotarsa decemlineata* (Chrysomelidae: Coleoptera) pupal fat body	EX-Cell 401TM	Long et al. (2002)
IPLB-Tconl	Hymenoptera	*Trichogramma confusum* (Trichogrammatidae: Hymenoptera) embryos	Ex-Cell 400	Lynn and Hung (1991)
IPLB-Tex2	Hymenoptera	*Trichogramma exiguum* (Trichogrammatidae: Hymenoptera) wasp embryos	Ex-Cell 400	Lynn and Hung (1991)
C7/10	Diptera	*Aedes albopictus* (Culicidae: Diptera) mosquito egg	Eagles MEM	Sarver and Stollar (1977)
59	Diptera	*Aedes aegypti* (Culicidae: Diptera) embryos	Ex-Cell 400	Peleg and Shahar (1972)
Line 2	Diptera	*Drosophila melanogaster* (Drosophilidae: Diptera) embryos and larvae	TC 199-M K	Schneider (1972)
UM-BGE4	Blattodea	*Blatella germanica* (Ectobiidae: Blattodea) embryo	UMN-B1	Ward et al. (1988)
AC20	Hemiptera	*Agallia constricta* (Cicadellidae: Hemiptera) embryos	TC 199-M K	McIntosh et al. (1973)

transferring cells from an older vessel to a new vessel containing fresh growth medium, allowing the cell line or cell strain to be propagated further (Goodman et al. 2001). When cells occupy all accessible substrates or when cells in suspension cultures exceed the medium's capacity to sustain further growth, cell proliferation is significantly slowed or entirely stopped. In order to maintain an optimal density for prolonged cell development and promotion of further proliferation, fresh medium must be introduced into the culture.

Several insect cell lines are established and are being exploited for their potential in cell molecular biology and virological research, industrial biotechnology, and insect pest management. Some of the insect cell lines that are being used for research are listed in Table 3.

Baculovirus–cell interactions

Baculoviruses infect midgut epithelial cells initially, and then the infection spreads to all the other tissues. This led to the evolution of two different kinds of virions with a major difference in the virion envelope structure. To survive the harsh alkaline environmental conditions in the midgut, occlusion-derived virions have a complex envelope that makes their penetration easier into the midgut (Rohramann 2019). Once the ODVs gain entry into midgut cells, they become budded viruses (BVs) to sustain in the neutral pH. These BVs are responsible for secondary transmission within the midgut and attack subsequently and spread throughout the entire body. At the late phase of infection, the BV levels are reduced, nucleocapsids interconnect with nuclear membranes and certainly become enveloped leading to the formation of virions, typically more than 30 virions found in one nucleus of AcMNPV (Miller et al. 1983). After the death, the caterpillar putrefies and releases the virions into the surroundings, if any other larvae feed on the contaminated leaves having these virions; once again the infection cycle starts by the release of ODVs by the virions into the midgut (Szewczyk et al. 2006). The infection cycle of baculoviruses is depicted in Fig. 1.

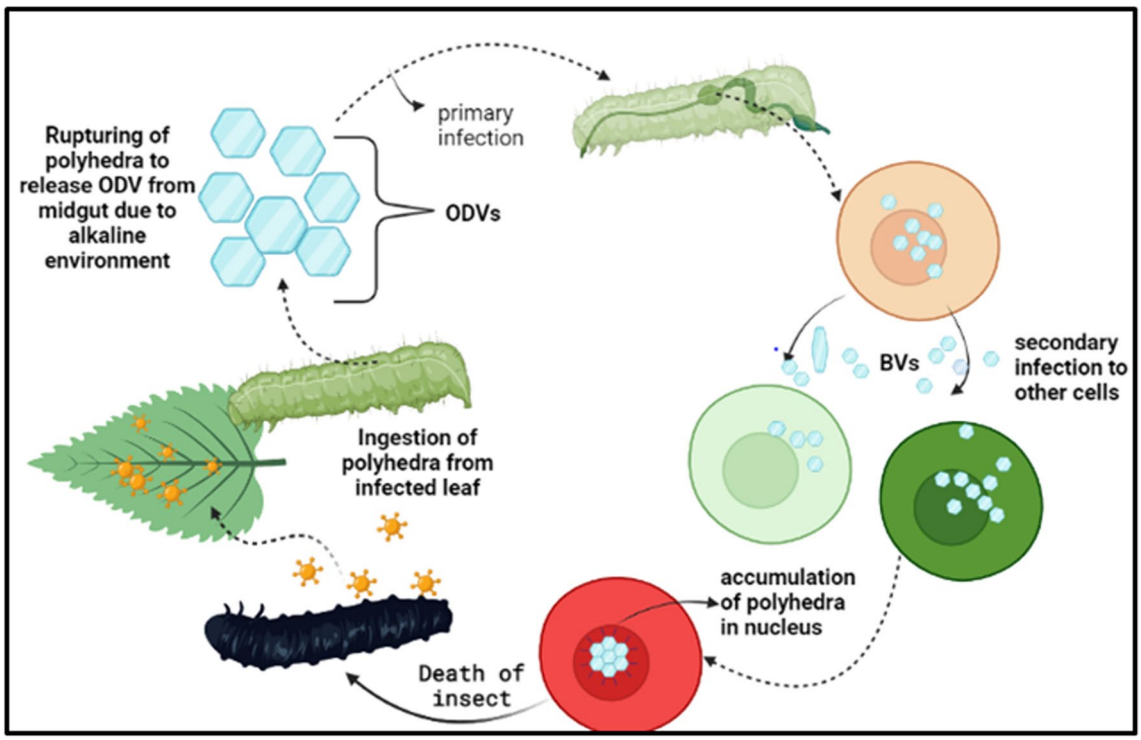

Fig. 1 Infection cycle of baculoviruses

BEVS

IC-BEVS is considered a low-cost and efficient protein factory (Rao 2003). Baculoviruses are DNA viruses, which are one of the most prominent viruses known to infect arthropods (Miller and Lu 1997); most of examples have been found infecting 600 species of insects belonging to different insect orders viz., Lepidoptera, Hymenoptera, Diptera, and Coleopteran, mainly on lepidopterans (Martignoni and Iwai 1986). These baculoviruses are excellent tools for recombinant protein production using insect cells. Baculoviruses are obligatory parasites with a narrow host range and are non-pathogenic to vertebrates, thereby making the baculovirus expression system the best platform for vaccines and therapeutics development (Felberbaum 2015). Using BEVS, heterologous proteins as well as virus-like particles (VLPs) can be generated in vitro. BEVS is currently being used for the production of VLP-based covid vaccines (Sacks 2021). A diagrammatic description of the mechanism in BEVS is depicted in Fig. 2.

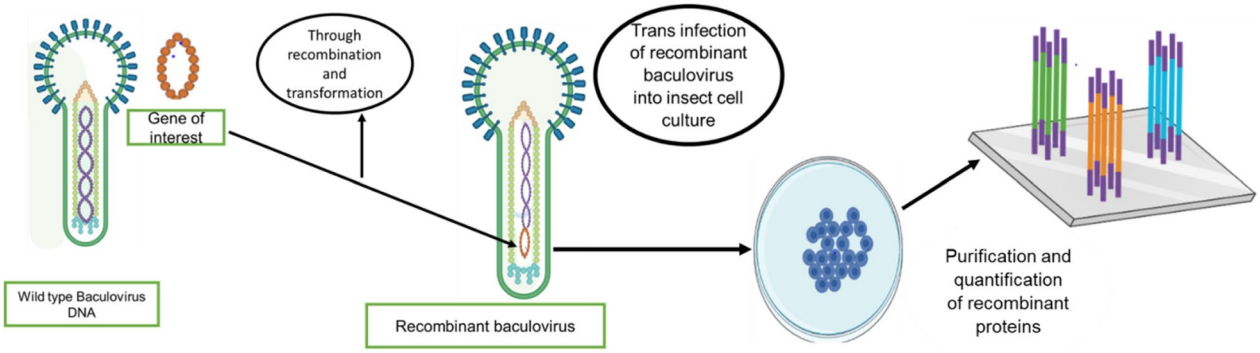

Fig. 2 A diagrammatic description of the mechanism in BEVS

Applications of insect cell cultures in pest management

Insect cell cultures are of immense potential in both the field of medicine and food production. One of the prominent applications is the utilization of the interaction between baculoviruses and insect cells for the production of recombinant proteins and vaccines (Mena and Kamen 2011), and insect cells can also be utilized for studying and understanding metabolic pathways and signaling mechanisms across cells (Pinto et al. 2019), screening for novel insecticidal targets in insect pests, analyzing the mode of action of potential insecticidal compounds (Mak et al. 2021), evaluating the toxicity of *Bt* insecticidal proteins and mass multiplication of entomopathogenic viruses (Wei et al. 2018), as a tool for studying virus transmission by insect vectors (Ghosh et al. 2020) and studying the physiology of insects at a molecular level (Arunkarthick et al. 2017). These various applications of insect cell cultures make them a practical tool for entomological research.

Table 4 Entomopathogenic viruses causing insect epizootics

Family	Nucleic acid	Target group	Example	References
Baculoviridae				
Nuclear Polyhedro Virus (NPV)	dsDNA	Lepidoptera	*Helicoverpa armigera* & *Spodoptera litura* (Noctuidae: Lepidoptera)	Prasad and Srivastava (2016)
Granulosis virus (GV)	dsDNA	Lepidoptera	*Cnaphalocrocis medinalis* & *Chilo infuscatellus* (Crambidae: Lepidoptera)	Harish et al. (2021)
Cytoplasmic Polyhedrosis Virus (CPV)	dsDNA	Lepidoptera	*Helicoverpa armigera* (Noctuidae: Lepidoptera)	Harish et al. (2021)
Pox Virus (PV)	dsDNA	Lepidoptera	*Amsacta moorei* (Erebidae: Lepidoptera)	Harish et al. (2021)
Ascoviridae				
Ascovirus	dsDNA	Lepidoptera	*Helicoverpa armigera, Spodoptera litura* & *Spodoptera frugiperda* (Noctuidae: Lepidoptera)	Harish et al. (2021)
Nudiviridae				
Nudivirus	dsDNA	Lepidoptera, Coleoptera	*Helicoverpa zea* (Noctuidae: Lepidoptera) & *Oryctes rhinoceros* (Scarabaeidae: Coleoptera)	Harish et al. (2021)
Iridoviridae				
Iridovirus	dsDNA	Lepidoptera, Coleoptera	*Galleria mellonella* (Pyralidae, Lepidoptera), *Phyllophaga vandinei* (Scarabaeidae: Coleoptera)	Jenkins et al. (2011), İnce et al. (2018)
Polydnaviridae				
Bracovirus	dsDNA	Lepidoptera	*Plutella xylostella* (Plutellidae: Lepidoptera)	Prasad and Srivastava (2016), Harish et al. (2021)
Parvoviridae				
Densoviruses	ssDNA	Hymenoptera, Hemiptera, Lepidoptera	*Galleria mellonella* (Pyralidae: Lepidoptera), *Myzus persicae* (Aphididae: Hemiptera), *Spodoptera frugiperda* (Noctuidae: Lepidoptera)	van Munster et al. (2003), Mutuel et al. (2010), Harish et al. (2021)
Reoviridae				
Cypovirus	dsRNA	Lepidoptera	*Helicoverpa armigera* (Noctuidae: Lepidoptera), *Bombyx mori* (Bombycidae: Lepidoptera) *Lymantria dispar* (Erebidae: Lepidoptera)	Li et al. (2006), Tan et al. (2008), Cao et al. (2012)
Rhabdoviridae				
Rhabdovirus	ssRNA(-)	Lepidoptera, Hemiptera	*Spodoptera frugiperda* (Noctuidae: Lepidoptera), *Nephotettix cincticeps* (Cicadellidae: Hemiptera)	Ma et al. (2014), Jia et al. (2021)
Dicistroviridae				
Dicistrovirus	ssRNA(+)	Lepidoptera, Hemiptera	*Helicoverpa armigera* (Noctuidae: Lepidoptera), *Rhopalosiphum padi* (Aphididae: Hemiptera)	Harish et al. (2021)

Mass production of entomopathogenic viruses

Insect pests cause devastating yield losses, and the chemical interventions are no longer effective as they were in the beginning; furthermore, their extensive usage led to many other problems rubbing salt into the wounds (Reddy et al. 2022). With this being said, integrated pest management strategies including biocontrol agents such as entomopathogenic viruses would be desirable for the pest management without causing any ecological backlashes. The majority of the viruses that infect insects are from the families Baculoviridae, Densoviridae, Entomopoxvirinae, and Reoviridae (Harrison et al. 2018; Harish et al. 2021). Different groups of viruses that cause epizootics in insects are listed in Table 4.

Mass multiplication of insect viruses that can be used for pest management is paramount for the integrated management of agricultural pests (Abd-Alla et al. 2020). Multiplying viruses using the insects in the laboratories is cumbersome, and the chance of being contaminated by other microbes is high; therefore, using the host insects for rapid multiplication of entomopathogenic viruses is not efficient and economical. Eventually, it was demonstrated that nuclear polyhedrosis viruses (NPVs) can replicate in insect cell lines (Vaughn 1981). So, this solves the problem as the cultured insect cells which are grown in culture media under aseptic conditions and can produce larger viral loads in a shorter amount of time and doesn't require much space (Agathos 2018). Moreover, quality control can be assured using this method. Cells derived from the Fall Armyworm, *S. frugiperda* (Noctuidae: Lepidoptera) are most widely used for the mass production of entomopathogenic viruses, especially the NPVs (Vaughn et al. 1977).

Evaluation and mass multiplication of cry toxins

For mass multiplication of *Bt* insecticidal proteins like the cry toxins, insect cell-baculovirus interactions are being utilized in BEVS to produce the toxins at a commercial scale (Del Rincón-Castro et al. 2011). Apart from their mass production, insect cell cultures can also serve as tools for understanding the mechanism of cry toxins and study the resistance developed in insects against these toxins at a molecular level. Generally, insect midgut epithelial cells are known to be the primary target for the *Bt* endotoxins. So, insect midgut cells should be cultured for studying the cytotoxicity of cry toxins in vitro. Midgut epithelial cell cultures of several lepidopterans and coleopterans were established and maintained in vitro for periods extending up to 3–6 months while preserving their differentiated characteristics (Smagghe et al. 2005). These midgut cell cultures were used for studying *Bt* endotoxin binding with the microvilli of intact epithelial cells from different lepidopteran species (Garcia et al. 2001). Technological tools like the patch-clamp technique and fluorescent probes were used to investigate the mode of action of *Bt* toxins on insect cells derived from different species and tissues (Gringorten 2001). The toxicity of Cyt2Ba, Cry4Aa, and Cry11A proteins was evaluated in IPLB-SF-21AE cells from *S. frugiperda* (Noctuidae: Lepidoptera), IPLB-LD-652Y cells from *Lymantria dispar* (Erebidae: Lepidoptera) BM-5 cells from *B. mori* (Bombycidae: Lepidoptera) and C6/36 cells from *Aedes albopictus* (Culicidae: Diptera) (Teixeira et al. 2012). *Spodoptera litura* (Noctuidae: Lepidoptera)-derived Sl-HP cells, Hi5 cells and Sf9 cells were used for evaluating the cytotoxicity of activated Cry 1Ac toxin to these different cell lines, and the results indicated that Sl-HP cells were most susceptible to Cry 1Ac (Chen et al. 2015).

Insect cell cultures are valuable tools for studying the causes of insect resistance to Cry toxins. Several theories for insect resistance against insect transgenics had been hypothesized, and one such is the alteration of toxin-binding site. Cry toxins were known to have certain specific receptors such as aminopeptidase-*N*, alkaline phosphatase, cadherins, ATP-binding cassette transporters which facilitate their cytotoxicity. Other possible mechanisms of resistance are altered processing of protoxins, enhanced immunity and midgut stem cell proliferation for rapid regeneration and replacement of cells damaged by cry toxins (Jurat-Fuentes et al. 2021). Using Sf9 cells, it was identified that scavenger receptor class C-like protein (Sf-SR-C) acts as a receptor for Vip3Aa protoxin in *S. frugiperda* (Noctuidae: Lepidoptera) (Jiang et al. 2018). ABCC2 proteins were identified as receptors for Cry toxins in *Bombyx mori* (Bombycidae: Lepidoptera) using Sf9 cells (Tanaka et al. 2013). Furthermore, the receptors for Cry2Ab toxins were found to be different from those of Cry1Ac based on binding profile evaluations in various insect cell lines (Wei et al. 2018). Insect cell cultures have significant potential for screening resistance against *Bt* transgenics. Mode of action of Vip3Aa insecticidal proteins was studied in Sf9 cells, and it was reported that the Vip3Aa toxin arrests cell cycle at phase, induces apoptosis in Sf9 cells, and also reduces the membrane potential and leads to mitochondrial dysfunction (Jiang et al. 2016).

Insect cell culture as a virological tool

Viruses are ultramicroscopic entities and cannot be seen with the naked eye. Yet, they are catastrophic in the way invade and hijack living organisms such that the entire world had no other option but to shut down to protect itself from a flu-causing virus, SARS-CoV-2 which plunged the world into an economic crisis through the Covid-19 pandemic (Ahmad et al. 2020). Plant viruses have a devastating effect on agricultural produce and

Table 5 Insect cell lines for virological research

Name of insect cell lines	Source	Applications	References
AC20	*Agallia constricta* (Cicadellidae: Hemiptera)	Virus infectivity tests	Chiu and Black (1967)
AFKM-On-H	*Ostrinia nubilalis* (Crambidae: Lepidoptera)	Useful in virus transfection research	Belloncik et al. (2007)
APE1	*Antitrogus parvulus* (Scarabaeidae: Coleoptera)	Development of engineered entomopox viruses as microbial control agents	Fernon et al. (1996)
AS-H 1	*Agrotis segetum* (Noctuidae: Lepidoptera)	Research on granulosis virus	Kozlov et al. (1990)
BCIRL/AMCYAgE-CLG	*Anticarsia gemmatalis* (Erebidae: Lepidoptera)	Embryos production of recombinant proteins and viral pesticides	Goodman et al. (2001)
BCIRL/AMCYAfO (/T)V-CLG	*Anagrapha falcifera* (Noctuidae: Lepidoptera)	Adult ovaries (/testes) fat body production of viral pesticides, mainly baculoviruses	Goodman et al. (2001)
BCIRL-Cc-AM	*Cactoblastis cactorum* (Pyralidae: Lepidoptera)	Support baculovirus infection and used in alternative biocontrol method	Grasela et al. (2012)
BTI-EAA	*Estigmene acrea* (Erebidae: Lepidoptera)	Larval hemocytes recombinant protein production insect cell lines	Hink et al. (1991)
CSIRO-BCIRL-HP1	*Helicoverpa punctigera* (Noctuidae: Lepidoptera)	Embryos useful in virological studies	McIntosh et al. (1999)
FPMI-CF-1	*Choristoneura fumiferana* (Tortricidae: Lepidoptera)	Midgut expression of recombinant proteins using baculovirus vectors	Hink et al. (1991)
FTRS-AoL	*Adoxophyes orana* (Tortricidae: Lepidoptera)	Neonate larvae research on viruses; susceptible to insect viruses	Mitsuhashi (1989)
High Five cells	*Trichoplusia ni* (Noctuidae: Lepidoptera) *ovarian cells*	Recombinant protein expression using baculovirus or transfection	Hink (1970), Zhang et al. (2008)
IBL-SLO-1A	*Spodoptera litura* (Noctuidae: Lepidoptera)	Useful in studies like replication of *S. litura* nuclear polyhedrosis virus in vitro	Shih et al. (1997)
IPLB-LD-64	*Lymantria dispar* (Erebidae: Lepidoptera)	Pupal ovaries used to quantitate infectivity	Goodwin et al. (1978)
IPRI 108	*Malacosoma disstria* (Lasiocampidae: Lepidoptera)	Larval hemocytes Nuclear polyhedrosis virus infectivity in vitro assay	Volkman and Goldsmith (1982)
KLBIQ-Chsu-I	*Chilo suppressalis* (Crambidae: Lepidoptera)	Production of insect virus expression vector	Liu et al. (2015)
LPC-Aa98-19	*Anacridium aegyptium* (Acrididae: Orthoptera)	Suitable for virus multiplication and manipulation and also used in biopesticides	Hernandez-Crespo et al. (2000)
Lub	*Lutzomyia longipalpis* (Psychodidae: Diptera)	Can be used in vaccines and diagnostic tests	Rey et al. (2000)
NIAS-LeSe-11	*Leucania separata* (Noctuidae: Lepidoptera)	Larval fat body research on nucleopolyhedrovirus	Yanase et al. (1998)
NIH-SaPe-4	*Sarcophaga peregrine* (Sarcophagidae: Diptera)	Used in study of host–parasite relationship in insect borne pathogenic microbes	Komano et al. (1987)
NIV-HA-197	*Helicoverpa armigera* (Noctuidae: Lepidoptera)	Embryonic tissue application in the mass production of this baculovirus as a bioinsecticide	Sudeep et al. (2002)
NN-1	*Nephotettix nigropictus* (Cicadellidae: Hemiptera)	Used in phytoreovirus research	Duan and Zhang (2014)
PLB-Ekx4T	*Ephestia kuehniella* (Pyralidae: Lepidoptera)	Embryos useful in biocontrol research and susceptible to nucleopolyhedroviruses	Lynn and Ferkovich (2004)
RAE25	*Rhipicephalus appendiculatus* (Ixodidae: Ixodida)	A useful tool in defining the complex nature of the host vector and pathogen relationship	Bell-Sakyi et al. (2007)
RIRI-BR1	*Blaps rhynchoptera* (Tenebrionidae: Coleoptera)	Used as folk medicine in Yunnan province China	Zhang et al. (2018)
Schneider 2 (S2)	*Drosophila melanogaster* (Drosophilidae: Diptera)	Useful in transfection research	Benting et al. (2000), Suske (2000)
Se6FHA	*Spodoptera exigua* (Noctuidae: Lepidoptera)	Useful in nuclear polyhedrosis virus research and the production of recombinant proteins	Hara et al. (1993)

Table 5 (continued)

Name of insect cell lines	Source	Applications	References
Sf9 cells	*Spodoptera frugiperda* (Noctuidae: Lepidoptera)	Pupal ovarian tissue recombinant protein production using baculovirus and in the evaluation of host–virus interaction	Davis et al. (1993), Ma et al. (2014), Wilde et al. (2014)
Sf21 cells	*Spodoptera frugiperda* (Noctuidae: Lepidoptera)	Pupal ovarian tissue research on baculoviruses and their use for producing recombinant proteins	Chen et al. (2005), Lynn (2003)
TI-1	*Thysanoplusia intermixta* (Noctuidae: Lepidoptera)	Studies of insect pathogenic viruses and baculovirus expression vector system	Hashiyama et al. (2011)
WIV-BS-02	*Biston suppressaria* (Geometridae: Lepidoptera)	Infection studies of homoceros tubulosa nuclear polyhedrosis virus	Mitsuhashi (1989)
WIV-BS-481	*Biston suppressaria* (Geometridae: Lepidoptera)	Larval hemocytes research on baculovirus studies	Grasela et al. (2012)

the losses instigated by them are estimated to be 30 billion US dollars annually (Rao and Reddy 2020). Studying viruses and their biology would help us devise strategies to assuage the yield losses caused by the insect viruses.

As we know, insect vectors play a pivotal role in the spread of plant viral diseases. Insect cell cultures could be game-changers as we deploy this novel technology for understanding the multiplication of plant viruses in the plant cells and how they are transmitted by insects. Given the size of viruses, it would not be easy to study their biology in insect vectors, but then cultured insect cells would avail us to inoculate with the viruses and study their replication at the cellular level. Cell cultures enable us to identify the site of virus accumulation, specificity of infection and replication of plant viruses in insect vectors (Adam 1984). Not only this, but they also help us to study the virus-receptor dynamics, the entry point of viruses, their multiplication inside cells, and their movement from one cell to the other (Wei et al. 2009, Mao et al. 2013, Chen et al. 2014). By identifying a suitable receptor that allows the virus to interact with the cells, insect-transmitted viral diseases could be prevented from spreading further using RNA interference (Kanakala and Ghanim 2016). Various cell lines of insect vectors used for studying plant viruses in vitro are listed in Table 5.

Insect cells as models for studying signaling mechanisms and metabolic pathways

Apart from entomological aspects, insect cell cultures could also be used for learning basic cell biological concepts, cell-to-cell signaling mechanisms, and identify metabolic pathways and their products as well using high-throughput screening (HTS) technology which is used for drug discovery. Established insect cell lines along with HTS platforms would revolutionize bioassays for compositional analysis of metabolic pathways in insects as well as other living organisms.

Trans-membrane protein, ASGP2 associates with ErbB2 receptor tyrosine kinase using Sf9 cells and a baculovirus expression system. ErbB2 receptor tyrosine kinase is pivotal in several developmental processes, and its overexpression could lead to the development of tumors. Using BEVS and Sf9 cells, it has been reported that ASGP2 modulates the activity of receptor tyrosine kinase (Carraway et al. 1999). Insect cells are very useful in studying the mechanisms of signal transduction as some of the signal transduction receptor proteins like the G-protein-coupled receptors are evolutionarily conserved across the various classes of living organisms (Broeck 1996). Insect cell lines Hi5 and Bm-5-based expression systems were used for expressing odorant receptors (ORs) from a mosquito, *Anopheles gambiae* (Culicidae: Diptera) which help identify their human hosts by responding to cues such as human sweat (Tsitoura et al. 2010). Studies like this could enable us in the near future to identify insect receptors that aid in identifying host plants based on odor cues. Sf9 cell-based expression systems are useful in analyzing mammalian receptors linked to Ca^{+2} signaling and homeostasis (Hu et al. 1994). PI3K/AKT/TOR pathway mediated autophagy induced by curcumin was elucidated in Sf9 cells (Veeran et al. 2019). Similarly, pathways related to the growth and development of insects can be understudied in vitro with the help of insect cell lines using the HTS platforms (Kayukawa et al. 2020).

Insect cell cultures for toxicity studies

Currently, pesticide formulations that target insect nervous system, muscular system, respiratory system and endocrine system are being used for pest control purposes. So, ascertaining physiological processes of insects at cellular level using insect cell lines not only helps us in understanding the cell biology and physiology but also enables us to devise novel pesticide molecules which will affect the life-sustaining processes of insects.

Insect cell cultures are also useful in ascertaining the mode of action of novel insecticidal compounds and also compare the toxicities of different compounds in both the target organisms and non-target organisms using their cell cultures (Salehzadeh et al. 2002). Insect cell lines are also used for high-throughput screening for novel insecticide targets in insects viz., endocrine targets, metabolic targets, neurological and muscular targets, ascertain the insecticide resistance mechanisms in insects, and evaluate insecticidal proteins from *B. thuringiensi* (Bacillaceae: Bacillales) (Mak et al. 2021). Toxicity of the insecticide bendiocarb was evaluated in both mammalian and insect cell cultures. Cytotoxicity was measured in terms of proliferation of cells and cytopathology in liver cells (WBF344) and kidney cells (RK13) of mammals, and Sf21 cells from *S. frugiperda* (Noctuidae: Lepidoptera). Proliferation in Sf21 cells was affected the most, while it was significantly reduced in both WBF344 and RK13 cells. Cytopathology was measured in terms of lactate dehydrogenase (LDH) concentration in the culture medium. Mammalian cells had relatively high leakage of LDH from the cells into the medium indicating cellular damage (Polláková et al. 2012). Insect cell cultures also have immense potential in the drug discovery and development of novel insecticidal formulations targeting novel sites in insect pests for pest management (Choi and Vander 2021).

Insect cell lines have additional applications as sentinels for testing environmental contaminants and their toxicity. Several industrial chemicals along with other pharmaceuticals are known to disrupt endocrine system of mammals. These pollutants are known to impair the health of humans (Darbre 2019). Insects are used as sentinels to observe the malefic effects of these pollutants on physiological processes that occur in humans as well as other higher animals (Wilson 2005). Insect cell lines are also used for testing the damage caused by environmental contaminants and evaluating the malign effects of these chemicals on endocrine system of vertebrates (Dinan et al. 2001).

Future prospects

Insect cell cultures might have a predominant role in entomological research in the near future. They are the cheapest protein factories for mass production of recombinant proteins and manufacturing of viruses (Ikonomou et al. 2003). Insect cell cultures can also be used to study the immune reactions of insects to biotic and abiotic stresses in vivo (Zhang and Turnbull 2018). They offer efficient model systems to interrogate drug delivery and metabolism. They can be used for evaluating the mode of action of insecticides and analyze novel target sites for chemical control (Wing 2021). They form the model systems for ascertaining insecticidal proteins and evaluating Bt-resistance mechanisms in insect pests (Teixeira et al. 2012). Apart from entomological research, insect cell lines could also be used for understanding basic cell biology, metabolic pathways, and effects of toxic and carcinogenic compounds like polycyclic aromatic hydrocarbons, tobacco-specific nitrosamines, Benzo [a] pyrenes, etc.

Conclusion

In the present review, an attempt was made to highlight the significant role of insect cell cultures in expediting entomological research and advancing the development of pest management strategies. The type of cells in an organism will determine its capabilities, and insect cell cultures will provide an insight into the mechanisms and machinery that made insects the most successful group of animals. A better understanding of the biology and the physiological processes will help us to devise tactics that could upgrade our arsenal in the fight against insect pests of food crops. Henceforward, insect cell cultures are likely to revolutionize the research in both the agricultural and medicinal sectors.

Abbreviations

ABCC2	ATP-binding cassette subfamily C member 2
AC20	*Agallia constricta*-derived cell lines
ATP	Adenosine triphosphate
BEVS	Baculovirus expression vector system
Bt	*Bacillu thuriengiensis*
BV	Budded viruses
CP-169	*Cydia pomonella*-derived cell line
CPV	Cytoplasmic polyhedrosis virus
DNA	Deoxyribonucleic acid
dsDNA	Double-stranded deoxyribonucleic acid
dsRNA	Double-stranded ribonucleic acid
ESF 921	Expression systems
ESF AF	Insect cell culture medium, animal-free
GV	Granulosis virus
HIV	Human immunodeficiency viruses
HTS	High-throughput screening
LD	*Leptinotarsa decemlineata*-derived cell line
LDH	Lactate dehydrogenase
MDBK	Madin-darby bovine kidney
MDCK	Madin-darby canine kidney
NN-1	*Nephotettix nigropictus*-derived cell lines
ODVs	Occlusion-derived virus
ORs	Odorant receptors
PI3K	Phosphatidylinositol-3-kinase
PV	Pox virus
RNA	Ribonucleic acid
SARS-CoV-2	Severe acute respiratory syndrome coronavirus 2
Sf-SR-C	*Spodoptera frugiperda* scavenger receptor class C-like protein
Sf9, Sf21	*Spodoptera frugiperda*-derived cell lines
SFM	Serum-free medium
ssDNA	Single-stranded deoxyribonucleic acid
ssRNA	Single-stranded ribonucleic acid
TI-1	*Thysanoplusia intermixta*-derived cell lines
Tn5, Tn368	*Trichoplusia ni*-derived cell lines
TOR	Target of rapamycine
VLP	Virus-like particles
VERO	Verda reno
Vip3Aa	Vegetative insecticidal proteins

Acknowledgements
Not applicable

Author contributions
JRB designed the project. JRB, KS, YG, KR, AG, and NN prepared the first draft text. SSRM guided and proof reading. All authors read and approved the final manuscript.

Funding
Not applicable.

Availability of data and materials
Not applicable.

Declarations

Ethics approval and consent to participate
Not applicable.

Consent for publication
Not applicable.

Competing interests
The authors declare that they have no competing interests.

Received: 31 May 2023 Accepted: 7 August 2023
Published online: 12 August 2023

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