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Promoting Efficacy of Human Platelet Lysate on Proliferation of Mammalian Cell Lines Used for Vaccine Production

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Abstract

The current study was conducted to determine to what extent human platelet lysate (HPL) can be used to enhance the growth of three cell lines used for propagation of Bovine Ephemeral Fever Virus (BEFV) and Rabies virus (RV) for vaccine production. HPL; Fetal Calf Serum (FCS) and Newborn Calf Serum (NBCS) were used as supplements to the used cell culture medium (Minimum Essential Medium "MEM") for passaging of three cell lines including baby hamster kidney (BHK-21); African green monkey kidney (VERO) and Madin Derby canine kidney (MDCK). Through three successive cell culture passages; for each cell line; it was found that HPL supported growth and propagation of BHK-21, VERO and MDCK cell lines without altering their morphologic and functional characteristics. It has been accepted as a suitable alternative to FBS and NBCS, and it can be used in vaccinal production.

KEYWORDS
Human Platelet Lysate (HPL), Growth factor, Cell culture.

INTRODUCTION

Animal cells, soft tissue, and organs are isolated from their hosts and grown in artificial environments to create cell cultures. Cell culture refers to maintaining and promoting the growth of cells in a suitable medium that closely resembles their natural environment. Numerous animal cell types, including tumour cells, muscle cells, epithelial tissues, and fibroblasts, can be fully developed in cultures (Merten, 2006). Using a convenient culture medium that contains nutritional components like serum, calcium ions, hormones, etc. to support normal cell growth, differentiation, and proliferation, it is feasible to cultivate animal cell cultures in vitro. Serum is crucial because it provides a source of growth and adhesion factors controls the permeability of cell membranes, and transports lipids, enzymes, micronutrients, and other components to the cell. However, there are several drawbacks to using serum, including its high cost and undesirable consequences, such as excessive stimulation or inhibition of cell growth and/or function (Oyeleye *et al*., 2016). Since its establishment, the cell culture technique has evolved into a crucial and important instrument for biological research, biotechnology, and pharmaceutical production facilities (antibodies, regenerative medicine, vaccination, and other therapeutic production) (Leist *et al*., 2008; Park and Eve, 2009). As a source of the hormones, growth factors, amino acids, proteins, vitamins, inorganic salts, trace elements, carbohydrates, lipids, etc. required for cellular metabolisms and cell growth, the media of this cell culture and its supplements play a crucial role (Yao and Asayama, 2016). Essential components found in foetal and newborn calf serum, such as serum albumin, fetuin (Fisher and Puck, 1958), hormones, vitamins, trace elements, growth factors, etc., are necessary for cell attachment, proliferation, and maintenance (Puck *et al*., 2010). Additionally, Human Platelet Lysate (HPL), which contains potent mitogens, contains platelet-derived growth factor, fibroblast growth factor, insulin-like growth factor, epidermal growth factor, and a number of other growth factors, including connective tissue growth factor, vascular endothelial growth factor, and chemokines, cytokines, and a variety of -granule factors (Burnouf *et al*., 2016). These factors are released by physiologically active platelets throughout the clotting process and support cell stemness, migration, adhesion, and proliferation (Rauch *et al*., 2011). The ability of HPL to maintain both suspended and anchorage-dependent cells has been amply proven (Johansson *et al*., 2003). When comparing the importance of foetal, newborn calf serum and HPL, for instance in the expansion of stem and progenitor cells *in vivo*, there are obvious benefits of HPL, such as cost effectiveness and highly standardized large-scale manufacture because it uses expired platelet concentrates. Additionally, higher cell proliferation in HPL culture allows for the timely and adequate synthesis of safe cell therapies. Contrarily, a risk-based approach must be used to evaluate the potential dangers of bovine serum, such as xeno-immunization or the transmission of bovine infections. For the manufacture of GMP-compliant cells, HPL has emerged as a new standard, especially for cell types for

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which serum-free fully defined medium are not yet available. In addition to foetal bovine serum, human platelet lysate (HPL) is an excellent cell culture media supplement for a range of different cell types. When used as a raw material, HPL enables humanised manufacturing of cell therapies within a reasonable amount of time while allowing animal serum-free cell propagation and extremely effective stimulation of cell proliferation. HPL suppliers must take special quality considerations for identity, purity, potency, traceability, and safety. Release criteria must be established to describe how well HPL batches can support a certain cell culture. Regulatory regulations state that the initial component for HPL preparation is fresh or old platelet concentrations from healthy blood donors. Individual platelet lysate units can be combined into a single HPL batch to balance donor diversity in crucial platelet-derived cytokines and growth factors. HPL safety will be further improved by the pathogen reduction technologies that are being used more and more (Oeller *et al*., 2021). Strong mitogenic and chemotactic effects are produced when growth factors from platelets are isolated. The growth factors are employed in numerous applications and diverse cell cultures to research their roles and the complexity of the associated signalling pathways. They have also been used on a number of cancer cell lines to study their impact on cancer cell growth, migration, and invasion. (Wakefield and Roberts, 2002; Fredriksson *et al.*, 2004; Van Horssen *et al.*, 2006). Additionally, it has been discovered that employing it has allowed for the successful long-term culture of a number of mammalian epithelial cells (Reddan *et al*., 1981).

The current research aimed to examine the impact of HPL on the growth patterns of the Baby Hamster Kidney (BHK), African Green Monkey Kidney (VERO), and Madin Darby Canine Kidney (MDCK) cell lines, as well as to assess the potential for their use in place of foetal and newborn calf sera to reduce the time and money needed for the production of the rabies and bovine ephemeral fever vaccines.

MATERIALS AND METHODS

Sera and growth factor supplements

Sera

Fetal calf and Newborn calf sera (Virus and Mycoplasma screened) supplied by Gibco, – USA were used in concentrations of 10% and 5% respectively as supplements for the growth cell culture media.

Growth factors

Lyophilized Human Platelet Lysates (HPL) were prepared according to the patented method designed by Prof. Dr Hossam M. Fahmy, Professor of Laboratory and Transfusion Medicine, Ain Shams Medical School, Cairo, Egypt.

The purified sterile growth factors were aseptically dispensed in sterile vials each containing a concentration of growth factors equivalent to that obtained from platelets coming from 20 ml of whole human blood, having a concentration of $1x10^9$ platelets/microlitre. Followed by a lyophilization step (El Samahy *et al*., 2021; El-Gohary *et al*., 2021). They were used in a concentration of 10% as a supplement for the growth of various cells in culture media

Cell cultures

Baby Hamster Kidney (BHK-21); African Green Monkey Kidney (VERO) and Madin Darby Canine Kidney (MDCK) cell lines were supplied by Veterinary Serum and Vaccine Research Institute (VSVRI), Abasia, Cairo, Egypt. These cell lines have been cultured at a density of 4.02×10^4; 3.3×10^4 and 3.8×10^4 cells / ml of BHK-21; Vero and MDCK respectively in 25 cm^3 tissue culture flasks using Minimum Essential Medium (MEM) supplied by Sigma Chemical Company, USA.

Each of BHK-21, VERO and MDCK was cultured supplemented with 5% Fetal calf serum, 10% Newborn calf serum and three different concentrations (5%, 7.5%, 10%) of the growth factors separately of each supplement. Such cell propagation was carried out each time in three tissue culture flasks and cell counting was carried out automatically at the duplicated times, which were 24, 48 and 72 hours post culturing for the MDCK, BHK-21 and VERO cell lines respectively and the highest cell count of each line obtained with the different concentrations of each supplement was determined.

Automatic live cell counting

To collect the grown cells in tissue culture flasks, trypsinization was done. Next, centrifugation at 2000 rpm for 10 minutes at a cold centrifuge was performed. Cell pellets were suspended in a predetermined volume of phosphate buffer saline (PBS) after the supernatant was discarded. An aliquot of the cell suspension was stained by mixing it 1:1 with a 0.4% Trypan blue solution, and 10 l of the stained cell suspension was pipetted into a counting slide, which was then placed inside the TC10 automated cell counter (Bio-Rad Laboratories, Inc.). This procedure counts the number of live cells. Then, the population doubling number (PD)—defined as the difference between the number of cells obtained from a given passage and the number of cells seeded in that passage—and the population doubling time (PDT)—defined as the typical amount of time required for a cell population to double—were computed as follows: PD = (Log (N2) - Log (N1)) / Log 2, where N1 is the initial cell count and N2 is the cell count following 100% confluence.

Where T is the incubation time in any units, PDT is defined as T Log2/(LogXe - LogXb).

The cell count at the start of the incubation period is Xb.

Xe is the number of cells after the incubation period.

Viruses

Local strain of Bovine ephemeral fever virus (BEFV/Abasia/2000) (Daoud *et al*., 2001) adapted on BHK-21 (Azab *et al*., 2002) with a titer of $10^{7.5}TCID_{50}$/ml and Evylene Rowateniski Ableth strain of rabies virus (ERA) were supplied by the Department of Pet Animal Vaccine Research (DPAVR), VSVRI and used for passaging in the mentioned cell lines under the effect of the used media supplements.

Virus titration

BHK-21 and MDCK; cell lines were used to titrate rabies virus & VERO cell line was used to titrate BEF virus using the microtiter technique according to Rossiter *et al.* (1985) and the virus titer was expressed as $\log_{10}$ $TCID_{50}$/ml using the formula of Reed and Meunch (1938).

Statistical analysis

Statistics were used to describe the data in terms of mean and standard error. An ANOVA test was used to compare more

than two variables. Using the analysis of variance for repeated measures and Bonferroni's multiple comparisons, the time course changes of each concentration were compared. Microsoft Excel spreadsheets version 10 and SPSS software for Windows version 25.0 (SPSS Inc., Chicago, IL, USA) were used to conduct the statistical analysis. It was deemed statistically significant at $P < 0.05$.

RESULTS AND DISCUSSION

The present obtained results tabulated in Table 1 confirmed that HPL supports growth and expansion of all cell lines, and there is no difference in terms of cell morphology in all cultivated cell lines. Although the cells cultured in presence of HPL experienced a less massive proliferation compared to those grown on FCS and NBCS. The results of the viability of the MDCK cell line showed a significant increasing ($p<0.001$) when used media supplement with the HPL 5and 7.5% than the used of 5% FCS, while it decreases when used a 10% HPL compared with the FCS, NBCS and other concentrations of HPL. While the viability of the BHK cell line showed a significant increasing ($p<0.001$), when used media supplement with the 5 % and 10% HPL compared with the FCS, and the 7.5% of HPL. The VERO cell line showed the same viability when supplement its media with 5%FCS or 5% HPL while the viability of the cell decreased in the other concentration of HPL than the 10% NBCS which gave the best viability result.

As demonstrated, each type of cell line requires a certain media, which has a big impact on how well its cell culture works. The best media for each cell line type must be discovered experimentally because different types of cell lines require very particular growth supplies.

The result of cell population doubling number (PD) of BHK cell line was slightly the same with the using of 5% FCS and 7.5%HPL while the other concenteration of HPL showed a significant decrease than in case with FCS and NBCS ($P<0.001$). The PD of VERO and MDCK cell lines showed a significant decrease with any concentration of HPL as supplement in their growth media than FCS and NBCS ($P<0.001$) and there is a significant increase in the doubling time (PDT) of BHK cell line when using the HPL as supplement in the growth media with a concenteration 5% and 10% by $P<0.001$and $P<0.05$respectively than the using 5% fetal calf serum while the 7.5% HPL as a supplement in the growth media gave the PDT as the cell growth with 5% FCS ,and other cell lines VERO and MDCK their PDT showed a significant increasing when using HPL than the FCS and NBCS ($P<0.01$ and $P<0.001$).

The obtained results are matching with those of Sangeetha Kandoi *et al.* (2018) who found that HPL is a viable alternative to FBS for generating clinically relevant numbers of MSC from explant cultures over enzymatic method, and agree with Mohamed *et al*. (2020) whose study supported that HPL supplement as a potential alternative for FBS to culture Vero and Hep-2 cells and with El-Dakhly *et al*. (2019) who used Equine lyophilized growth factor in cultivation of different cell lines.

HPL promote the replication and propagation of rabies and BEF viruses in BHK-21; VERO and MDCK cell line when used as a supplement in the used media as well as FCS & NBCS. The titer of these viruses in different cell line with different media supplement represent in table 2 and these results agree with the results obtained by Ojha *et al*. (2019), who mentioned that active platelets release proteins and cytokines including CXCL4 (PF4), CCL5, and fibrinopeptides that control the human infection of numerous pathogenic viruses like HIV, H1N1, and HCV. They looked at how platelets affected DV replication as well as that of a closely related Japanese encephalitis virus (JEV), as platelet activation is a defining feature of infection with the Dengue virus (DV).

Table 1. The effect of different media supplement on the growth rate of different cell lines.

Type of cell line		Type and concentration of different media supplements				
		Fetal calf Serum 5%	Newborn Calf Serum 10%	Human Platelet Lysate (HPL)		
				5%	7.50%	10%
BHK-21	*PDT*	6.75±0.12	5.86±0.05	7.50±0.06***	6.76±0.05	7.07±0.06^{*,a}
	PD	7.34±0.09	8.24±0.11	6.45±0.02^{b}	7.20±0.05	6.79±0.05^{b,c}
	Viability (%)	78.85±0.15	86.50±0.50	85.9±0.20^{d}	76.5±1.10^{e,f}	83.85±0.45$^{d,g,h,\#}$
VERO	*PDT*	8.51±0.04	8.20±0.57	10.51±0.58**	10.58±0.01**	9.38±0.50$^{\$,@}$
	PD	8.58±0.08	8.28±0.07	6.74±0.07^{b}	6.81±0.01^{b}	7.47±0.18^{b,l}
	Viability (%)	89.75%±0.35	97.90%±0.40	89.85±0.45^	73.80±0.40^{b}	87.75±0.55^
MDCK	*PDT*	2.90±0.03	3.13±0.09	4.66±0.10***	4.41±0.06***	5.52±0.125$^{***,\&}$
	PD	8.23±0.04	7.76±0.12	5.31±0.05^{b}	5.43±0.06^{b}	4.46±0.01^{b,m}
	Viability (%)	65.60±0.70	91.05±0.45	85.86±0.46***	78.13±0.17***	51.95±0.65^{b,m}

$^{*}p<0.05$ significant increase than FCS;$^{**}p<0.01$ significant increase than FCS and NBCS.
$^{***}p<0.001$ significant increase than FCS and NBCS; $^{a}p<0.001$ significant `increase than NBCS; $^{b}p<0.001$ significant decrease than FCS and NBCS; $^{d}p<0.001$ significant increase than FCS ; $^{c}p<0.05$ significant decrease than FCS; $^{f}p<0.001$ significant decrease than NBCS; $^{g}p<0.05$ significant decrease than NBCS; $^{h}p<0.001$ significant increase than 7.5% HPL; $^{\#}p<0.05$ significant decrease than 5% HPL;$^{\$}p<0.05$significant increase than NBCS; $^{@}p<0.05$significant decrease than 7.5% HPL;$^{l}p<0.01$ significant increase than 5% and 7.5 % HPL; $^{b}p<0.001$ significant decrease than FCS and NBCS;$^{^}p<0.001$ significant decrease than NBCS; $^{\&}p<0.001$ significant increase than 5% and 7% HPL; $^{m}p<0.001$ significant decrease than 5% and 7.5 HPL.

Table 2. ERA and BEF virus titers in cell lines using different media supplimented.

Titrated VIRUS	Virus titer (($log_{10}TCID_{50}$/ml) in used cell line								
	BHK			VERO			MDCK		
	FCS	NBCS	HPL	FCS	NBCS	HPL	FCS	NBCS	HPL
ERA	7.6	7.5	7.6	6.4	6.3	6.4	6.8	6.8	6.6
BEF	7.4	7.2	7.1	6.5	6.4	6.2	-	-	-

*FCS: Foetal Calf Serum; *NBCS: Newborn Calf Serum; *HPL: Human Plate Lysate

CONCLUSION

It has been demonstrated that HPL supports the growth and propagation of BHK, VERO and MDCK cell lines without altering their morphologic, phenotypic, and functional characteristics. It has been accepted as a suitable alternative to FCS and NBCS and it can be used in vaccine production.

CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

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