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IMPACT OF POLYETHYLENE GLYCOL ON NEWLY ESTABLISHED OVARIAN CELL LINE (SL-OMI) FROM LEAF WORM OF *SPODOPTERA LITTORALIS* AND ON VIRAL INFECTION

[36]

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ABSTRACT

New cell line from the Egyptian cotton leaf worm (*Spodoptera littoralis*) originated from the insect ovaries tissues was established, cloned and maintained as contentious cell line. Nine serial dilutions of Polyethylene glycol (PEG) were prepared from mother suspension (18.2g PEG_{8000} in 100 ml double distilled de-ionized water) started from10^{-1}-10^{-9}. Each concentration was prepared to treat five replicates of C35 cell dish. Treated cells were observed daily and counted 5 times within 15 days, and cell viability was detected by using the viability staining (Trypan blue). Mother suspension treatment reduced cell number 30 minutes to 24 hours post application. The concentrations of 10^{-6}, 10^{-5} and 10^{-7} PEG which were selected by the screening test were the most effective concentrations on increasing cell numbers respectively but the concentration of 10^{-6} was the most effective comparing with the untreated control. All concentrations from 10^{-3} to 10^{-9} supported cell growth rate more than control non-treated cells. PEG concentrations from10^{-5} to $1o^{-9}$ proves that PEG improve the cell proliferation and decrease cell death .An infection with Sl NPV baculovirus (Nucleopolyhedrovirus) was carried out on (SL omi) new established cells in the presence of PEG treatments which demonstrate that PEG has affected the permeability of cells to the NPV infection and enhances the viral replication.

Keywords: cotton leaf worm – cell culture – NPV – cell line.

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INTRODUCTION

Polyethylene glycol (PEG) is a straight-chain polymer of a simple repeating unit, induces macromolecular crowding of solutes in aqueous solution (Thermo Fisher Scientific Inc. and its subsidiaries 2009) and has a range of uses in molecular cloning, including: Fusion of cultured cells or with bacterial protoplast **(Charles, 2005)**, and precipitation of DNA molecules according to their size **(Hemant *et al.*, 2001; and Allen 1993).** The main goal was to prove the effect on the cells and on the viral replication. Successful NPV replication leading to productive infection proceeds through several sequential steps which include entry of virions into host cell, early viral gene expression, assembly and release of budded viruses(BVs), very late gene expression, and crystallization of polyhedrin into polyhedra which occlude virions with a phenotype different from that of BVs. Such an NPV replication cycle may abort at any step after viral entry into host cells **(Yohko *et al.*, 1997and Yasuhiro et *al.*, 2006).** The AcMNPV attachment to insect cells is receptor mediated via glycoproteins components, and it may even utilized host histones during at least the early stages of viral RNA and DNA synthesis **(Mettenleiter, 2002)** but **Wilson and Miller (1987)** reported alternatively that, the virions components responsible for cellular DNA synthesis inhibition may bind to or interact with host cell DNA polymerases in some cell lines and inhibit the activity, in present study we deal with the cell membrane as a barrier and used poly ethylene glycol as a fusion inducing agent which fuse and crowd the cells together.

MATERIALS & METHODS:

Preparation and screening test of the PEG appropriate concentrations for Sl cell line treatment:

Nine serial concentrations of Polyethylene glycol (PEG 8000) which is typical for common fusion technique used in mammalian cell culture **(Koichi Kato *et al.*, 2003; Huang Ming-His *et al.*, 2004)** were prepared from mother suspension (18.2g PEG in 100ml double distilled de-ionized water) then autoclaved for 20 minutes at121°C, the pressure was released under slow exhaust and cooled to

45°c in water bath, 18.8 ml of grace medium were added and swirled to mix, then it was kept at room temperature under check for one week for contamination with daily observation. Nine serial dilutions of PEG were prepared from mother suspension 10^{-1} to10^{-9}. Each dilution volume was prepared for five replicates, treatments were carried out on C35 culture dishes (3.5cm in diameter small plastic Petri dishes) contains 2ml media $2x10^5$ cells . Cells were daily observed and counted 5 times within 15 days. Cell viability was detected by using the viability staining (Trypan blue). The mother suspension effect was observed and photographed under the inverted microscope at 450X magnification during the first three hours at the same field every 30 minutes.

2 –Effect of selected PEG concentrations on the new *S L* cell line:

This test was depending on the results of the previously mentioned screening test. The most effective dilutions were selected (10^{-5}, 10^{-6} and 10^{-7}) and treated cells were observed, counted daily for two weeks and were photographed under inverted microscope at 450X magnification.

3- Impact of selected PEG concentrations on *Sl* NPV infected new *Sl omi* cell line:

Cell culture dishes were treated with the PEG concentrations, the zero time for each cell dish were counted. Treatments were divided into 3 groups (**a, b** and **c**) each group in 5 replicate:

a- Negative control: Non-treated cell line.

b- Positive control: 1-Treated cell line with virus (purified virions).

2-Cells treated with PEG (selected concentrations of PEG $10^{-5,}$ 10^{-6}, and $10^{-7.}$

c- The treatments:

cells + PEG 10^{-5} + Virus.

cells + PEG10^{-6} + Virus.

cell + PEG10^{-7} + Virus.

Diagnostic test for Sl-NPV detection on Sl-Omi new cell line by NPV nucleic probe and hyperdization tests:

Dot blot hybridization technique was a highly specific and effective technique to detect viral DNA replication in the tested cellular systems, depending on a highly specific molecule which is the viral nucleic acid. Labeled viral nucleic probe was constructed

using Boehringer Mannheim Kit (DIG-DNA Labeling System) prepared from SlNPV. Samples were probed with the viral DNA 10 &15 days post infection in both pellet and supernatant.

RESULTS & DISCUSSION:

Effect of the selected concentrations of PEG on Sl-omi cells in presence of 10% fetal bovine serum:

Results shown in **Table (1)** demonstrate that the mother suspension of PEG reduced cell number 24 hours only post application. The concentrations of 10^{-6}, 10^{-5} and 10^{-7} were the most effective concentrations on increasing cell numbers respectively but the concentration 10^{-6} was the best one comparing with the control. All concentrations in between 10^{-3} to 10^{-9} supported growth rate more than control. PEG concentrations in between 10^{-5} to $1o^{-9}$ proves that PEG improve the cell proliferation and decrease cell death semilary to the results which was stated **by (Jae *et al.*, 2002; Nakajima and Ikada, 1995)** on animal cell culture **(Figure 1,2).**

All concentrations (10^{-1} to 10^{-10}) affected cell proliferation more than the control non-treated cells after 24h, 4 days, and 9 days, but the control cells were more viable and their number were higher than the treated cells with the concentrations of PEG 10^{-1}, 10^{-2}, 10^{-3}, and 10^{-4}. The PEG concentrations of 10^{-9}, 10^{-8} seems to be effective after 24 hours post treatment but the number of cells decreased comparing with the Control 5 days post treatment. Mother suspension was fetal for cells, as it showed in the number of cells and in **Figure (1).** PEG concentration of 10^{-6} is the most effective concentration that affects positively the number of cells and keeps cells active for long time than any other concentration, this study could recommend the10^{-6} concentration for keeping cells more viable for longer time than control.

Effect of selected PEG concentrations on Sl omi cells treated in media free serum:

Jae *et al.* (2002) Stated that the Ac NPV-NOV maximum titer reached in the supernatant of serum free-cell cultures was higher than the maximum titer measured in the supernatant of serum supplemented cultures, this agrees with **Alice *et al.* (1973)** who studied AcMNPV

on different cell lines and found that the titer of virus was induced with serum free-medium. So in our study it was found that the combination between PEG and serum-free medium was effective similarly to 10% supplement FBS when using the same concentrations of PEG the results comparing with the control non-treated showed that the dilutions of 10^{-4}, 10^{-5} and 10^{-6} were the effective conc **Figure (4)** to enhance cell proliferation (data not demonstrated in details).

Polyethylene glycol as a factor enhances the NPV baculovirus in insect cell culture:

To understand and correlate the impact of PEG on NPV cell infection, the mechanism by which polyethylene glycol (PEG) mediates cell fusion has been studied by examining the movements of membrane lipids and proteins, as well as cytoplasm markers, from erythrocytes to monolayer of cultured cells to which they have been fused, this results which assumed by **Yoshito and Nakajima (1995); Wojcieszyn (1981);** they explained that in the presence of both fusogenic and non fusogenic PEG membranes are brought together at closely apposed contact regions while fluorescent lipid probes quickly spread from the membranes of erythrocytes to cultured cells in the presence of both fusogenic and non fusogenic PEG, and the proteins of the erythrocyte membranes were never observed to diffuse into the cultured cell membrane when the water-soluble proteins did not diffuse from the erythrocyte interior into the target cell cytoplasm until the PEG was removed. These data suggest that the coordinate action of two distinct components is necessary for fusion as mediated by PEG. Presumably, the polymer itself promotes close apposition of the adjacent cell membranes but the fusion stimulus is provided by the additives contained in commercial PEG **(Wojcieszyn, 1981).** The results shown in **Table (3)** and **Figures (5, 6, 7)** indicates that a certain concentrations of PEG especially 10^{-6} enhanced the viral production demonstrated by the number of PIBs /cell and showing the difference between the yield of viral occlusion bodies (polyhedra PIB) in Sl-Omi infected cells with NPV treated with the three different concentrations of PEG, which prove that using of Polyethylene glycol is effective

tool for virus replication in the attached monolayer cells. This data was confirmed by the detection of viral concentrations and replication by the dot blot hybridization technique which indicates in **Figure (8)** that the concentration of PEG 10^{-4} detect the highest concentration of virus in treated cell.

The nucleic probes indicated negative results in non treated cells,PEG10^{-6}, PEG10^{-7} and PEG 10^{-5}. and showed a positive results with purified virions of treated virus, PEG10^{-7}+virus, PEG10^{-5}+virus, and PEG10^{-6}+virus but it was noticed that PEG 10^{-6}+virus was the darkest dot reaction which prove that it contains viral DNA more than other concentrations.

Table 1. Number of Trypan blue stained viable and dead cells treated with 10 serial concentrations of Polyethylene glycol.

PEG Con.	Number in million of viable cells/mL				Number in million of dead cells/mL			
	24 hours	5 days	10 days	15 day	24 hours	5 days	10 days	15 day
10^{-3}	0.36	0.28	2.00	1.84	0.16	0	1.32	0.68
10^{-4}	0.56	0.52	1.8	1.6	0.6	0.2	1.08	0.76
10^{-5}	0.44	0.48	3.2	3.00	1.2	0.24	0.68	0.8
10^{-6}	1.2	3.04	3.8	3.12	0.16	0.36	0.44	1.2
10^{-7}	1.00	2.28	3.6	2.8	0.56	044	0.6	1.4
10^{-4}	0.72	1.16	1.92	1.96	0.56	0.48	1.24	1.6
10^{-3}	0.68	1.2	1.6	1.44	0.16	0.44	1.56	2.16
10^{-2}	0.76	2.36	1.24	0.96	0.56	0.48	1.56	2.2
10^{-1}	0.92	1.8	0.8	0.68	0.6	0.72	1.8	2.4
m.4	0.24	0.2	0.064	0.06	1.08	1.00	2.2	4
control	0.2	1.04	2.2	1.2	0	0	0.68	1.4

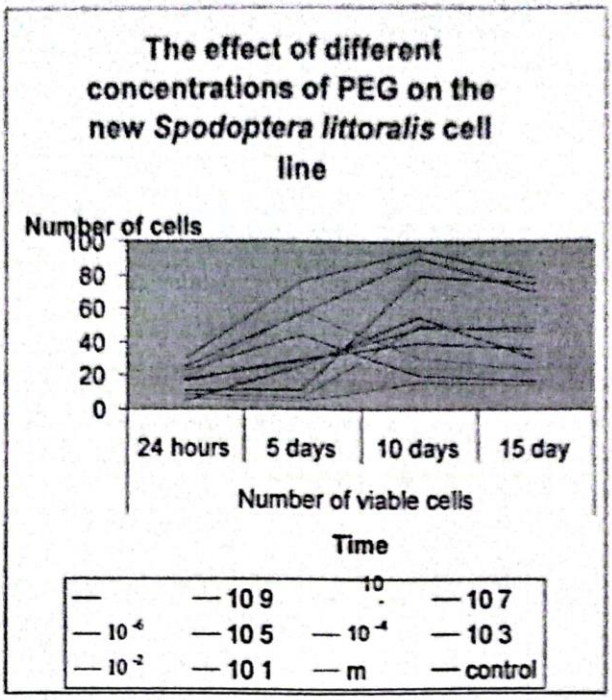

Figure 1. The effect of different concentrations of PEG on the viability of the new SI cell line.

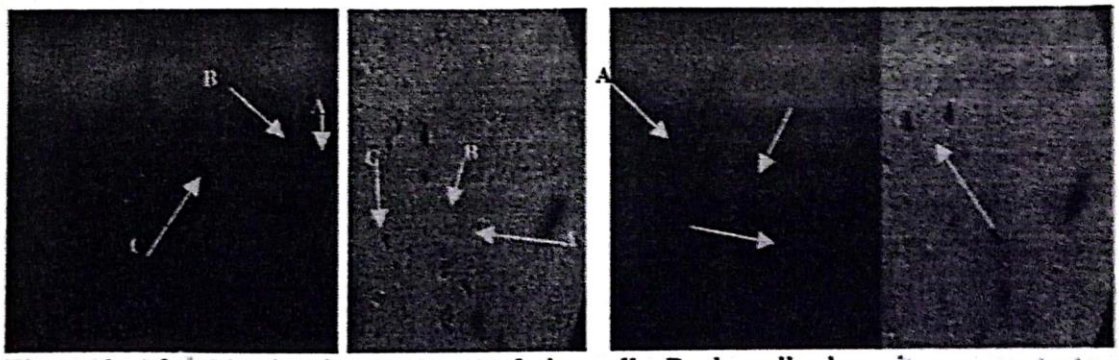

Figure 2. After 30 min of treatment: A- fusion cells, B- the cell release its own content, c- two cells start to fuse. Fig (2): the same field on Fig. 1 but after one hour: A- the fusion getting closer, B- the release of cells component become bigger, c- cell fusion, D- bud appear on cell. Fig. (3): After one hour and 30 min: A- two cells start to fuse, B and C – buds appear on cells. Fig. (4): The same field 3 but after three hours: A- the two cells become fussed as it is one big.

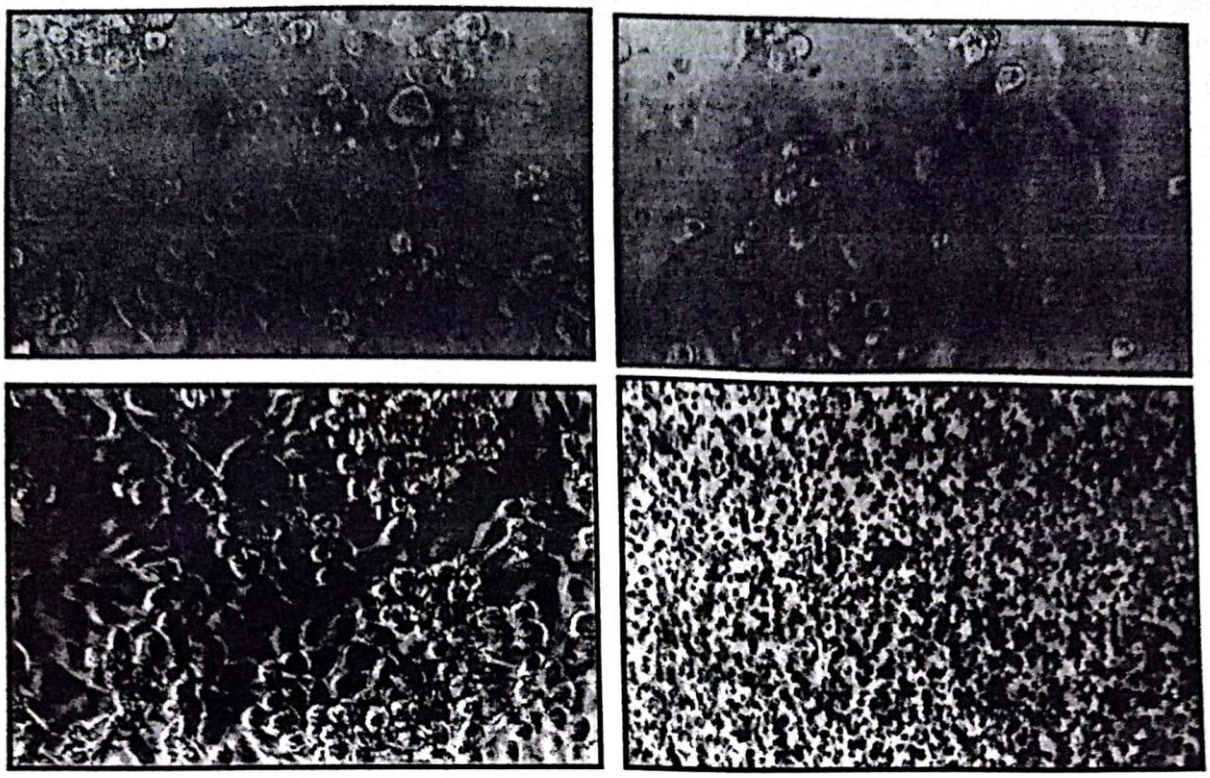

Figure 3. The concentration 10-6 found to be giving a very high density of cells, almost twice than in control, beside the cells were healthy and long active and viable more than any other concentration.

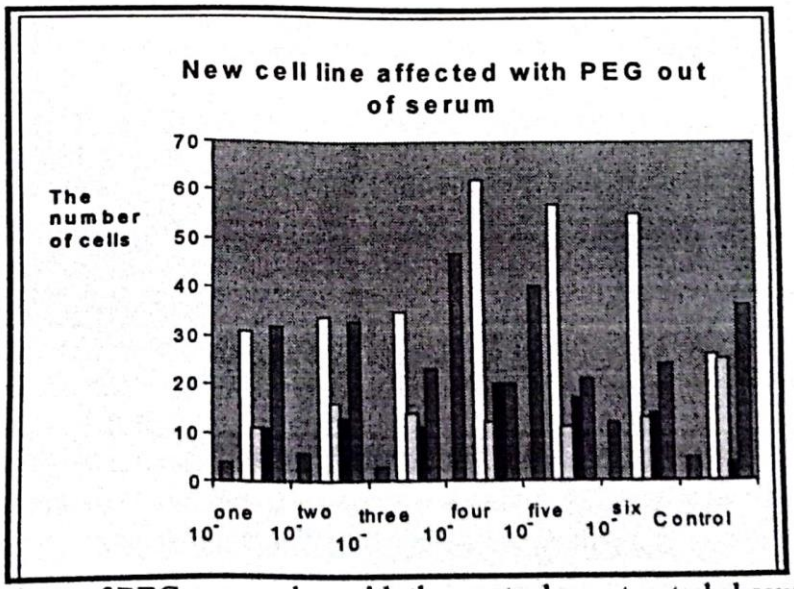

Figure 4. Concentrations of PEG comparing with the control non-treated showed that the dilutions of 10^{-4}, 10^{-5} and 10^{-6} were the effective conc.

Table 3. The effect of PEG concentrations on virus production on Sl-Omi new cell line.

PEG conc	number of viable cells				
	5 days	10 days	15 day	20 day	25 day
control	2.16	4.24	4.26	3.96	3
10-5	3.48	3.96	4.44	6.12	4.8
10-6	2.8	7.36	6.08	5.24	5.48
10-7	2.28	4.2	4.52	4.92	3.64
virus	2.12	3.88	3.6	3.32	2.28
vp7	2.28	4.6	5.08	5.36	4.48
Vp5	3.32	5	4.48	3.68	3.48
Vp6	1.92	3.4	3.88	5.76	4.88

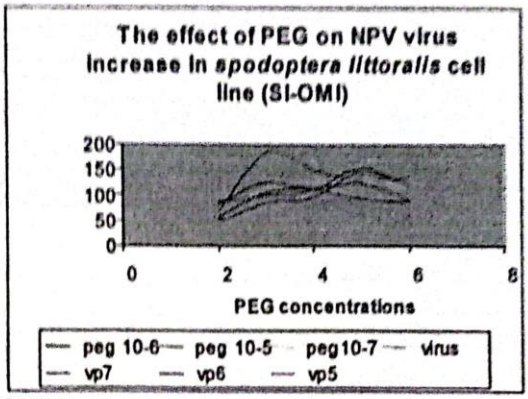

Figure 5. The effect of PEG concentrations on virus production on Sl-Omi new cell line

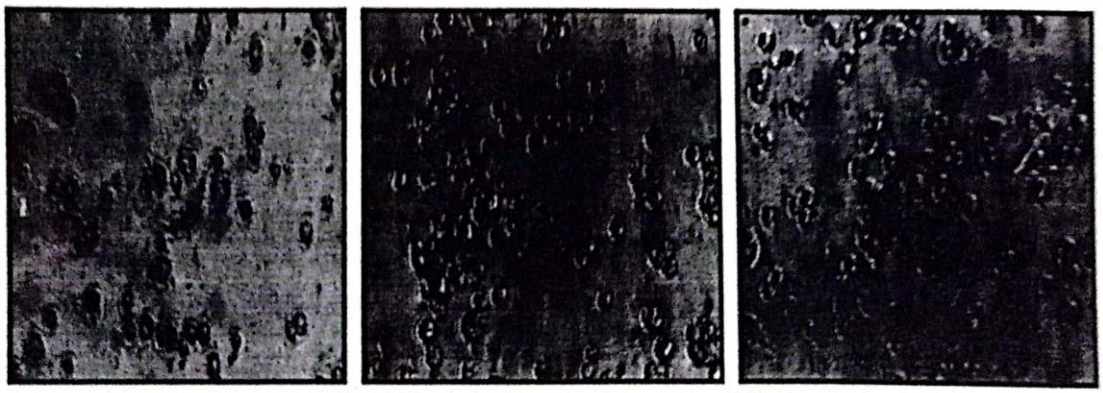

Figure 6. Effect of the selected PEG concentrations on the new *Spodoptera littoralis* cell line Sl-Omi infected with Sl-NPV.

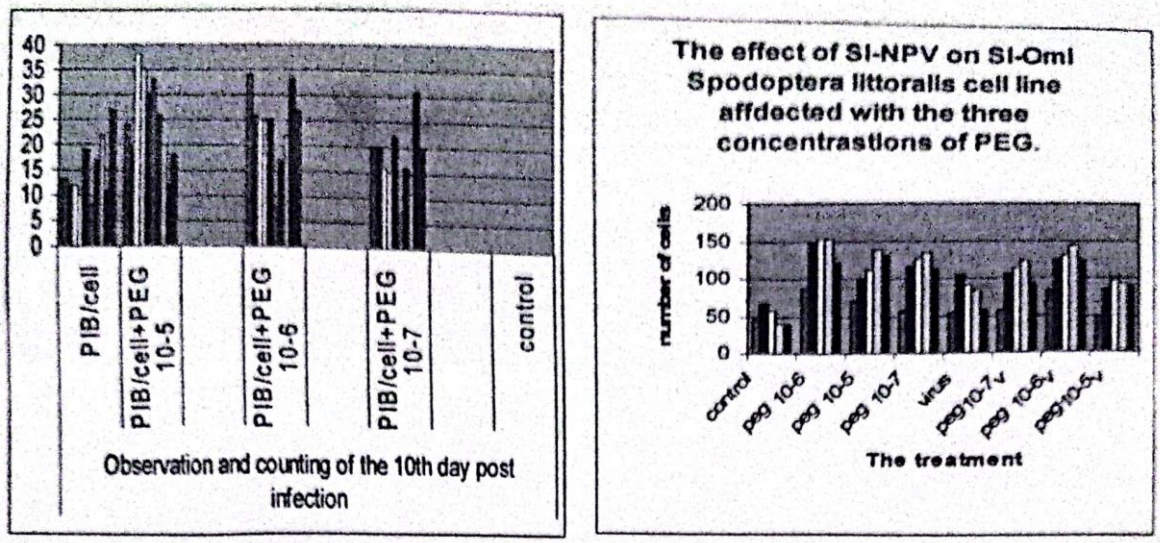

Figure 7. Observation and counting of polyhedra in ten nuclieus at the 10th (a) day post infection.

Table 4. Observation and counting of polyhedra in ten nuclieus at the 10th&15th day post infection.

Repl.	Observation and counting of the 10th day post infection				
	PIB/cell	PIB/cell+PEG 10^{-5}	PIB/cell+PEG 10^{-6}	PIB/cell+PEG 10^{-7}	control
1	34	24	38	20	
2	26	20	24	20	0
3	25	18	32	16	0
4	18	25	21	18	0
5	25	30	16	25	0
6	8	23	14	8	0
7	17	16	25	17	0
8	22	20	12	22	0
9	33	12	5	31	0
10	27	18	17	27	
replicates	Observation and counting of the 15 day post infection				
	PIB/cell	PIB/cell+PEG 10^{-5}	PIB/cell+PEG 10^{-6}	PIB/cell+PEG 10^{-7}	control
1	20	8	11	17	0
2	31	11	12	25	0
3	32	12	23	18	0
4	21	17	41	12	0
5	27	16	18	6	0
6	18	10	32	22	0
7	27	9	18	31	0
8	41	6	12	25	0
9	31	22	24	15	0
10	16	11	12	11	0

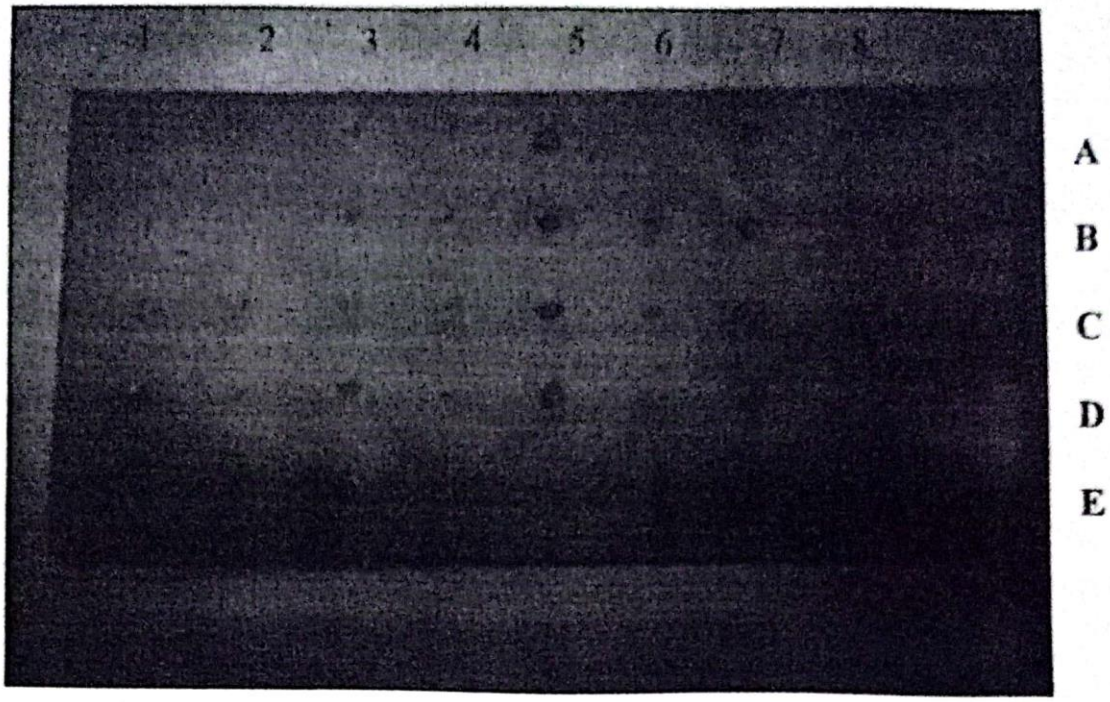

Figure 8. Dot blot hybridization membrane tested with nucleic probe indicated negative results in non treated cells (8), PEG10^{-6} (1), PEG10^{-7} and PEG 10^{-5}. and showed a positive results with purified virions(7), PEG10^{-7}+virus, PEG10^{-5}+virus, and PEG10^{-6}+virus but it was noticed that PEG10^{-6}+virus was the darkest.

CONCLUSION

The presence of PEG enhanced the SL-NPV virus replication in cells, The PEG concentration 10^{-6} was the most effective concentration, then 10^{-5} ,10^{-7} respectively ,the figures showing that the NPV infected cells become attached as a connected tissues which is prove that fused cells with PEG are more able to be infected (ROBINSON et al 1979). The concentration of 10^{-6} could enhance cell proliferation in high density of cells, almost twice than in control, beside the cells were healthy and viable more than any other concentration. In addition the concentrations 10^{-5}, 10^{-7} showed a good result to induce and enhance cell proliferation but less than 10^{-6} . The results in this study explained and demonstrated by the figs. and tables could agree with the results suggested in different type of vertebrate cells, cell fusion did not come to completion immediately

upon contact with PEG solution **(Nakajima and Ikada, 1995)** but gradually continued during post-incubation. The frequency of membrane fusion increased with increase in the cell density seeded in the culture plate and time of exposure to the PEG solution. Further, with increasing PEG concentration the frequency of fusion markedly increased, regardless of the PEG molecular weight **(Kristina Martinelle *et al*, 2010).**

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