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הקיטנגו היגולונומיא ,היגוליבורקימל הקלחמה
תואירבה יעדמל הטלוקפה

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Final report Bio-Fence

Objective: Evaluation of the antiviral activity (virucidal efficacy) of Bio-Fence surfaces against HSV-1.

Material and Methods:

In these experiments, we used Vero cells, african green monkey kidney cells, as host cells for the virus infection. The cells were grown in RPMI medium with 10% new born calf serum and antibiotic mixture (penicillin, streptomycin and nystatin) and incubated at 37^{o}C in a humidified air containing 5% CO2.

For these experiments, we used a stock of herpes simplex virus type 1 (HSV-1) [10^{8} plaque-forming units (PFU)/ml].

All experiments were done based on ISO 21702 (Even part of them are more stringent compared to ISO conditions).

Toxicity examination

As a first step it is important to examine possible toxicity of the various surfaces to Vero cells in order to be able to make the antiviral examinations. 400µl of culture medium was placed on each of the tested surfaces, incubated on the surface for different periods of time (1 and 24h). Then, mixed well on the surface and Vero cells were treated with various dilutions (1:1, 1:10, 1:1000) of this medium. The toxicity was tested by:

1.Direct count. The cells were counted by Neubauer hemacytometer indicating their replication rate.

2.Morphological changes were observed daily by optical inverted microscope (for 3 days).

Antiviral activity examination

Different amounts (50, 100, 200 and 400µl) from each of 3 concentrations of the virus (10^7, 10^6 and 10^5) were placed on the appropriate Bio-Fence surface as small drops (each drop about 3µl size) as can be seen in picture 1, stored inside Petri dish at room temperature for various periods of time (15min, 1h. and 24h). Then, 50µl of medium were placed on the drop area, mixed well and 50µl of this mixed medium were taken for infecting Vero cells monolayers (in 24 well plates) at 4 serial dilutions. The amount of infective viruses were determined by plaque assay.

Picture 1

Results

Toxicity

All the different examined surfaces did not show any toxicity, the number of the treated cells with the media which incubated on the surfaces was identical to the control untreated cells. Also there was no morphological changes in the treated cells compared to the control.

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Antiviral activity

The results presented in Table 1 and Fig. 1 are average of all the repetitions and also for the highest concentration of virus used for the experiment. The lower concentrations of the virus gave lower amounts of plaques as expected.

Table 1. Effect of the various Bio-Fence surfaces on HSV-1 infectivity after 1h incubation on these surfaces at room temperature.

Surface	**Amount of plaques (PFU/ml x 10^4)**					
	HSV-1 (10^7PFU/ml)					
	15min.		**1h**		**24h**	
	Drop (µl)		**Drop (µl)**		**Drop (µl)**	
	50	**100**	**50**	**100**	**200**	**400**
Control	14.2±0.6	19±0.9	11±0.7	16±0.9	22±1.5	48±2.7
0004-P	14.5±0.6	18±0.8	0	0	0	0
0001(WBM)	13.6±0.6	17±0.8	0	0	0	0

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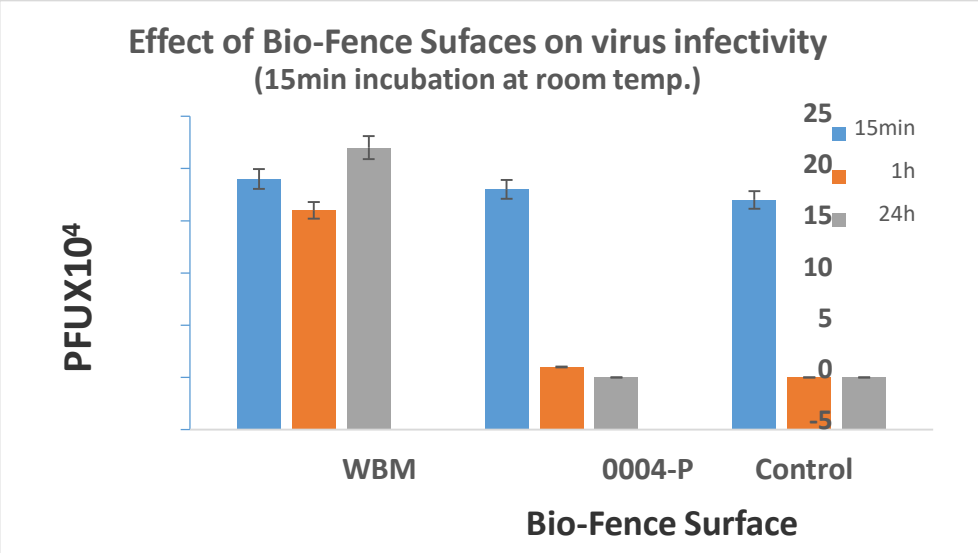

Fig. 1

The results of the viral test showed that both coated surfaces [0004-P and 0001(WBM)] completely eliminated the virus after **1 or 24h incubation** at room temperature even at the highest concentration I used (about 10^7 plaque forming units/ml) compared to the control which showed a very strong infection. However, it can be seen from the presented results that there was no effect on the viral infectivity after a **15min** of incubation at both tested surfaces.

These results can be seen in the following pictures of infected Vero cells with HSV-1 after incubation at the different surfaces for the different times of incubation.

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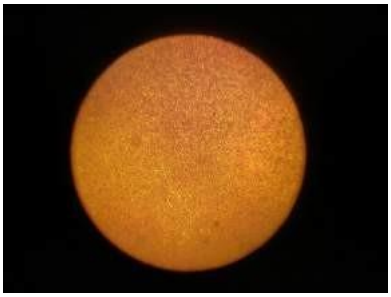
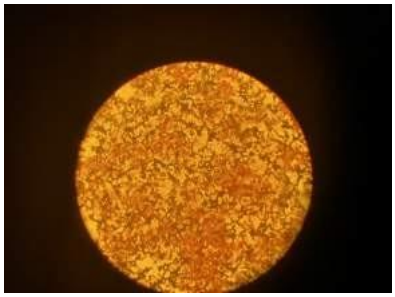
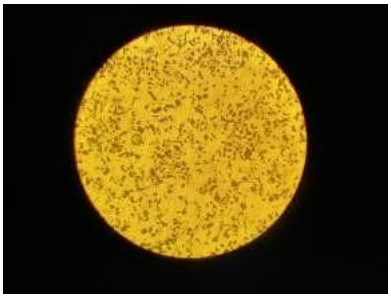


Control uninfected Vero cells

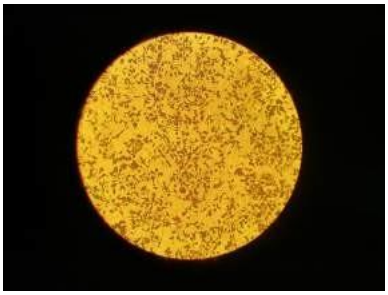


Infected Vero cells with HSV-1

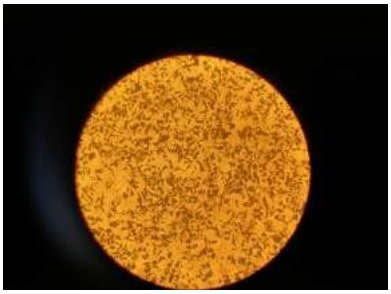
15 min incubation at room temp.



Infected cells with HSV-1 which incubated on Control surface



Infected cells with HSV-1 which incubated on 0004-P surface



Infected cells with HSV-1 which incubated on WBM surface

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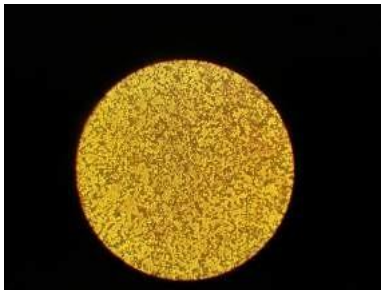
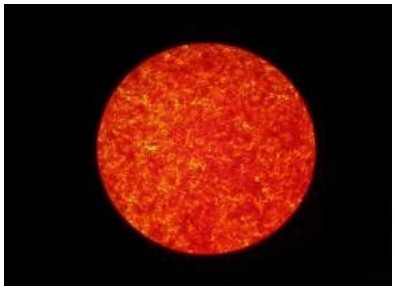
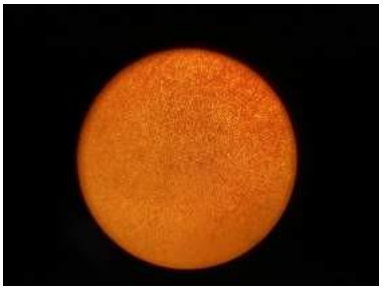
1h incubation at room temp.



Infected cells with HSV-1 which incubated on Control surface

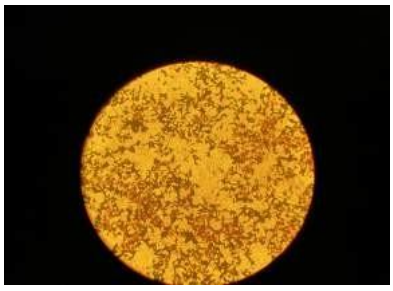


Infected cells with HSV-1 which incubated on 0004-P surface

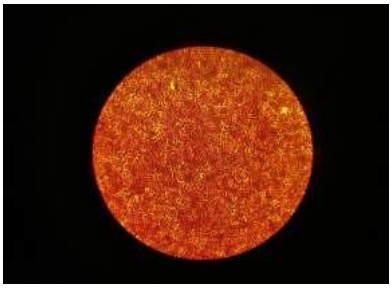


Infected cells with HSV-1 which incubated on WBM surface

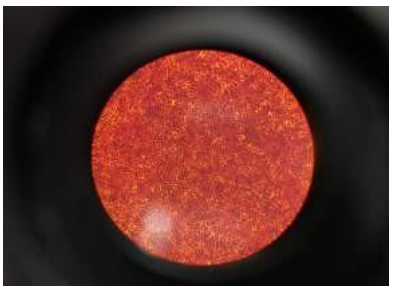
24h incubation at room temp.



Infected cells with HSV-1 which incubated on Control surface



Infected cells with HSV-1 which incubated on 0001-M surface



Infected cells with HSV-1 which incubated on WBM surface

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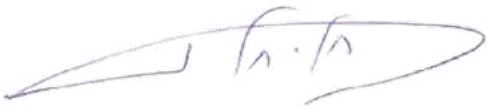
Conclusions

1. In light of these results, I declare that these coated surfaces are strongly inhibit the infectivity of HSV-1 after **1 or 24 hour** incubation on these surfaces at room temperature. Although we used in these experiments very high amount of virus (about $4x10^6$ PFU on about $1cm^2$ of the surface), these coated surfaces almost completely neutralized the viral infectivity even after 1h of incubation at room temperature.
2. HSV-1 belongs to the herpes viruses family which are enveloped DNA viruses and considered relatively as resistant to environment conditions. It is most likely that the antiviral activity of the tested coated surfaces is not specific to the herpes viruses and this activity seems to be directed to the envelope of these viruses. It is known that the envelope of enveloped viruses (which is similar to animal cell membrane) has viral glycoproteins, which called spikes and are responsible for the infectivity of the virus. Any damage to these spikes leads to a complete loss of the viral infectivity.
3. According to the mentioned above it is most likely to speculate and suggest that these coated surfaces will be highly effective against the infectivity of the corona viruses due to the fact that also corona viruses are enveloped viruses similarly to the herpes viruses.

Best regards

Prof. Mahmoud Huleihel



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