

STUDY OF THE KINETIC OF THE BIMOLECULAR INTERACTION "HOST-CELL-HERPES SIMPLEX VIRUS" IN THE CONDITION OF ONE STEP VIRUS GROWTH INFECTION PROCESS

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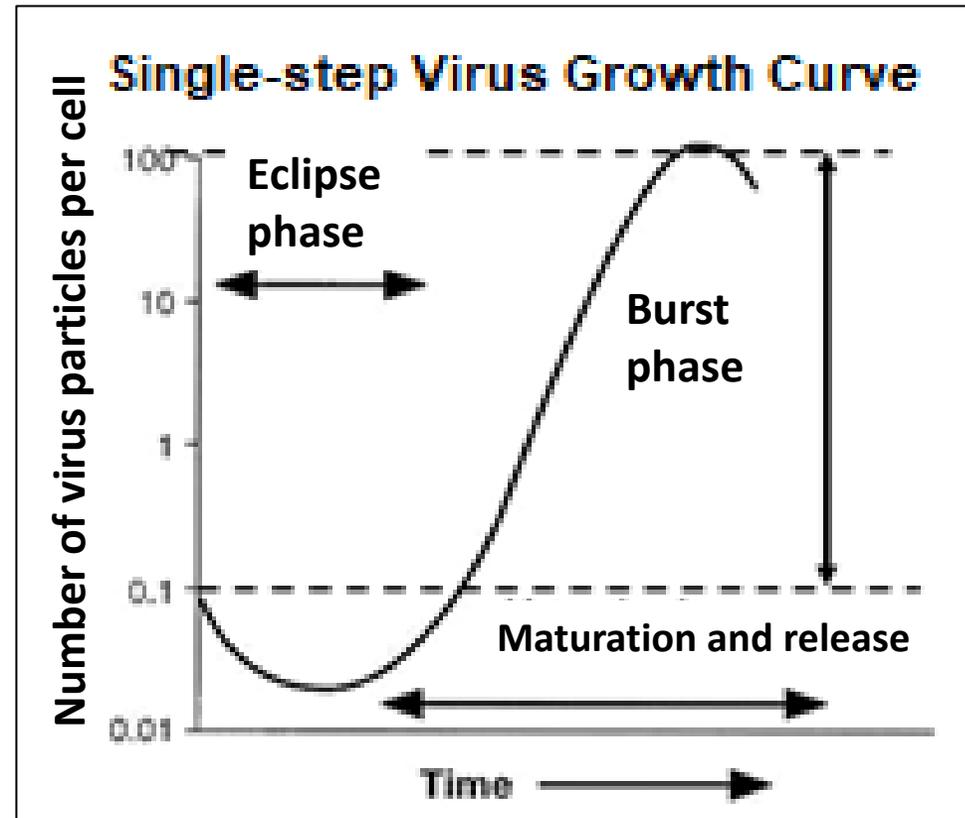
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Near 90% of infectious diseases are with viral etiology. Viruses are obligate intracellular parasites, replicating in living cells. Virus and cell form a complex called Virus – Host-cell (VC). In this bio complex, host-cell provides her machinery (structures and metabolism) to produce more viruses. Virus changes cell's metabolism and morphology (CPE).



Eclipse: no virus is recovered during the replication and assembly phases

Maturation and release: virus particles are made and can infect other cells

Burst size: the number of infectious viral progeny from a single round of replication

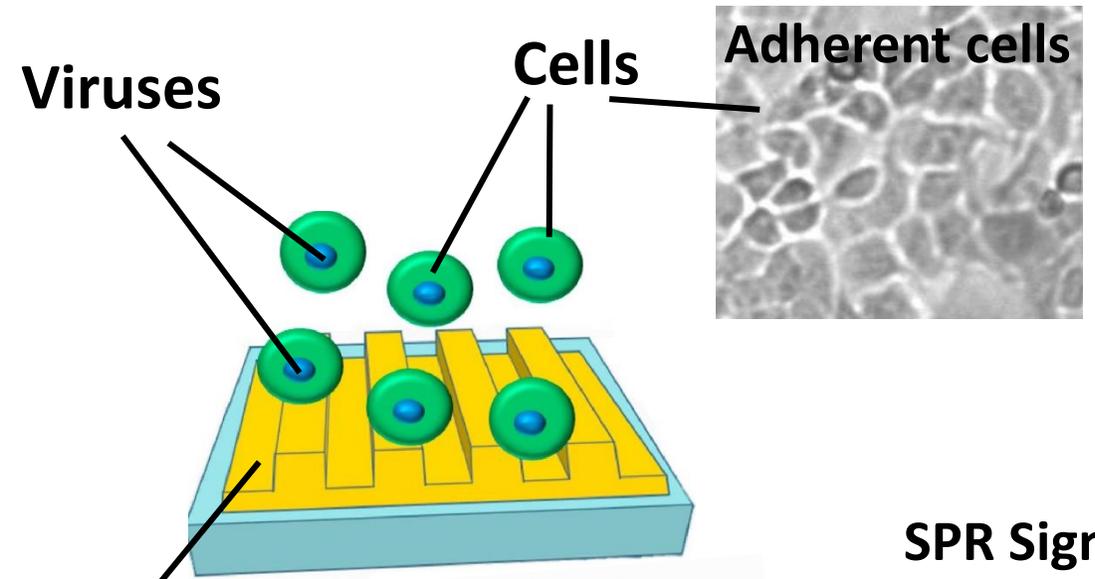
SUBSTANCES ANTIVIRAL SCREENING PROTOCOLS THROUGH CELL VIABILITY

Methods to analyze Cell Viability

MTT Test	Neutral Red	Crystal Violet	Trypan Blue	Cell Morphology

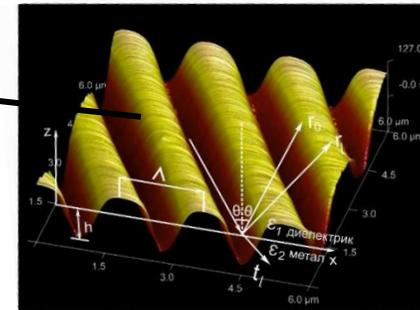
Time consuming and difficult to implement!

The aim of the present study is to detect and to evaluate the kinetics of a biomolecular **“host-cell—HSV-1” interaction** in a condition of one-step virus growth infectious process using the **Surface Plasmon Resonance (SPR) method** at different multiplicity of infection (MOI) and time of exposure.

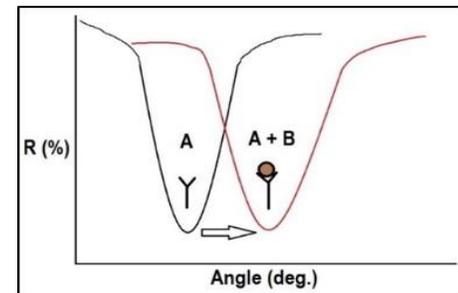


Is there a better method?

Sensor gold surface



SPR Signal



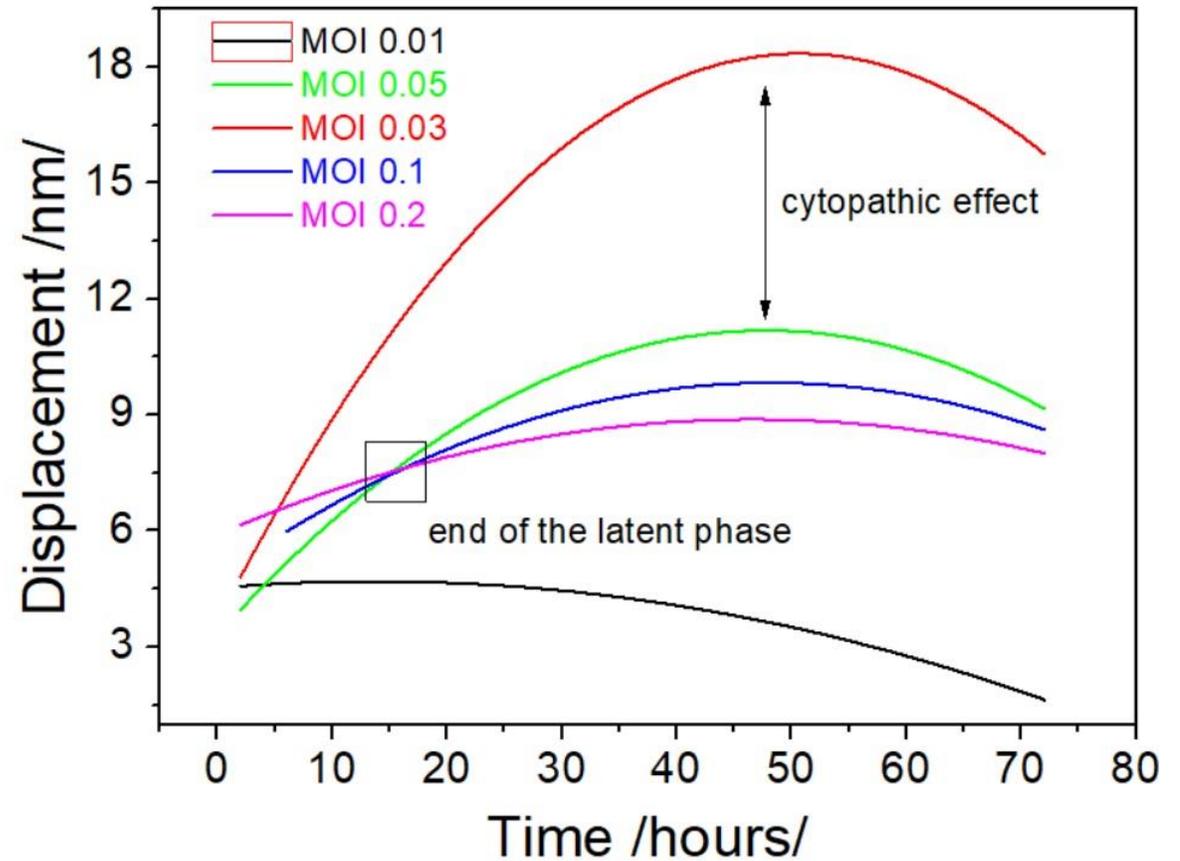
Materials

Cell culture	LEP –Human Embryonic lung cells
Employed viruses	HSV-1 clinical isolate
Cultural media	DMEM (Dilbecco's Modified Eagle's) supplemented with 2% fetal bovine serum (2% FBS) and antibiotics
Substrate	Gilded diffraction grating

Methods

Cell culture cultivation	Temperature - 37°C , 5% CO ₂ Passaging - 1:3-1:5 with density 2x10 ⁵ cell/ml
Substrate inoculation	Cells – 3 x 10 ³ cells/ml Virus - HSV-1 Multiplicity of infection (MOI): MOI = 0.01; 0.03; 0.05; 0.1; 0.2
Substrate cultivation	Temperature - 37°C , 5% CO ₂ Readout - 2, 6, 12, 24, 48, 72 hour after infection
Methods	SPR, MTT and morphology observation of cell-monolayers

RESULTS



The individual stages of the viral replicative cycle generate a SPR signal, which allows them to be recognized.