INFLUENCE OF NANOCOMPOSITES BASED ON CERIUM AND LANTHANUM OXIDES DOPED WITH SILVER ON HERPES VIRUS REPRODUCTION

Artiukh L.O.[1], Povnitsa O.Y.[1], Zahorodnia S.D.[1], Zahornyi M.M.[2]

[1]Zabolotny Institute of Microbiology and Virology, NAS of Ukraine, Kyiv, Ukraine

[2]Institute for Problems of Materials Science, NAS of Ukraine, Kyiv, Ukraine

E-mail: bilyavskal@ukr.net

Introduction

Viral infections are one of the principal causes of mortality in the entire world that result in significant human, social, and economic costs. Nanomaterials have been explored in medical applications, such as in the treatment of cancer and pathogenic disease, leading to new protection strategies against bacteria and viruses. It is found that noble metal/metal oxide and mixed metal oxide nanocomposites (NCs) show excellent antiviral properties (fig.1).

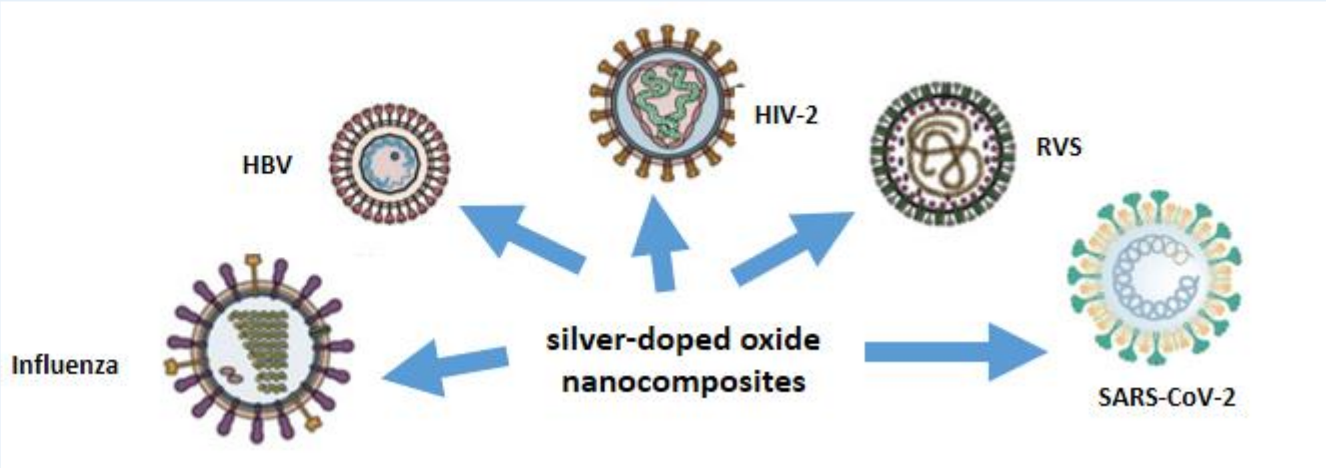

Figure 1. Antiviral efficacy of silver-doped oxide nanocomposites

The **aim** of this study was to investigate antiviral properties of nanosized composites based on cerium and lanthanum oxides doped with silver.

Materials and Methods

Synthesis of the composite powders containing lanthanum, cerium, and silver was performed via the co-precipitation method. The weight ratio of argentum was set in the range from 0 to 5 wt.%. The morphological and optical properties and the structure of the prepared nanocomposites were analyzed using X-ray diffraction, scanning electron microscopy with EDX, and nitrogen adsorption-desorption based on BET and Raman spectroscopy.

The Syrian hamster kidney fibroblasts (BHK-21) and the strain US of herpes simplex virus type 1 (HSV-1/US) were used in this study. The cells and virus were cultured according to standard methods.

Cell viability after NCs exposure was assessed using a dyer 3-(4,5-dimethylthiazolo2-yl)-2,5-diphenyltetrazolium bromide (Merk, Darmstadt, Germany) (MTT). Cell viability (%) under the treatment of NCs in comparison to control cells (100% viability) was calculated. To investigate the best order of nanocomposites antiviral effects, four experimental procedures were used as follows (fig.2 a, b). The HSV-1 titers were quantified by the TCID method.

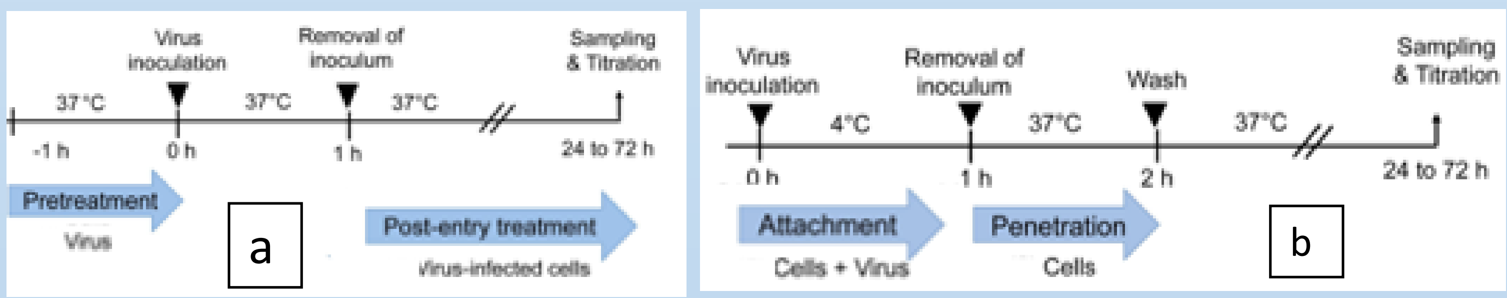

Figure 2. Time-of-addition experiment.

a-Pretreatment: virus are treated with an NCs before virus inoculation to the cells. Post-entry treatment: virus-infected cells are treated with an NCs. b-Blockade of attachment: cells and viral inoculum are treated with an NCs at 4 °C. Blockade of penetration: cells inoculated with virus at 4 °C are treated with an NCs at 37 °C.

Results

Characteristics of metal oxide nanopowders doped with silver

As shown in Figure 3 (1-a,b), the obtained homogeneous particles are not perfect spherical shape, and aggregates size of cerium dioxide is large compared to lanthanum oxide particles. Analysis of the EDS spectra of composites CeO_2-Ag and La_2O_3-Ag shows follows, wt.%: 77.14 Ce, 4.36 Ag, 18.5 O and 76.38 La, 6.07 Ag, 17.55 O. According to X-ray phase analysis, the primary particle size of pure cerium dioxide and CeO_2-Ag composite are 7 nm. For lanthanum oxide, the size of the primary particles is 27 nm, and for the composite La_2O_3-Ag (>5 wt.%) it increases to 35 nm.

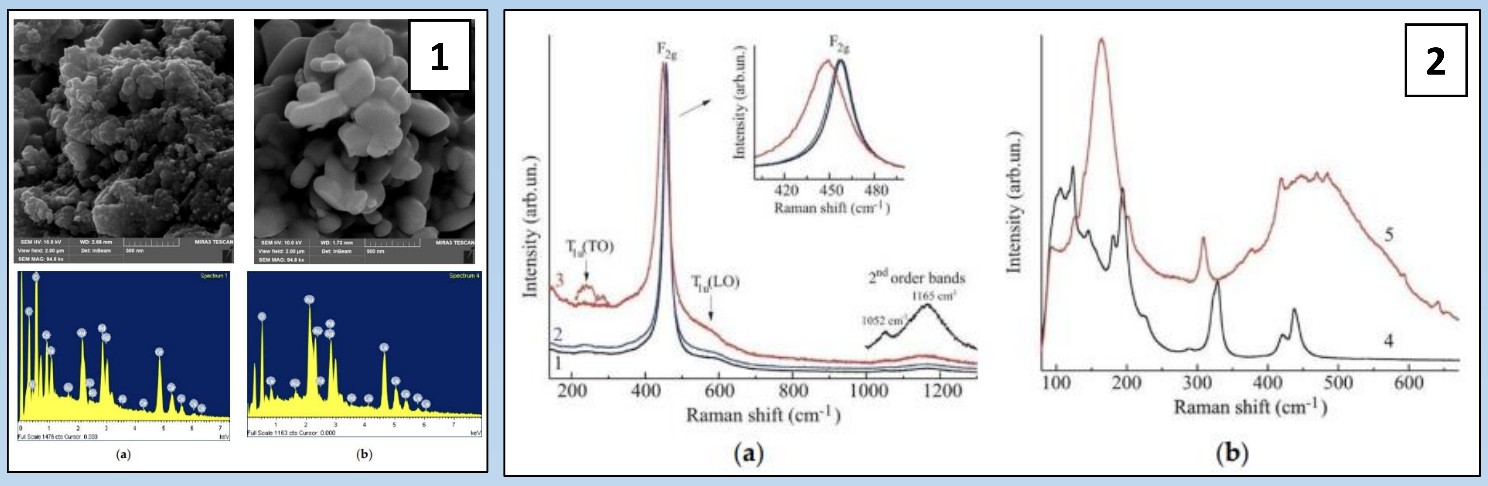

Figure 3. The physicochemical characteristics of metal oxide nanopowders doped with silver. **1 -** SEM-EDS analysis of the surface of nanocomposite powders: (a) CeO_2–Ag (4 wt.%); (b) La_2O_3-Ag (5 wt.%). **2** - Raman spectra of (a) nanocomposite based on CeO_2: 1-undoped CeO_2; 2-CeO_2-Ag(2 wt.%); 3-CeO_2-Ag (4 wt.%); (b) nanocomposite based on La_2O_3: 4-undoped La_2O_3; 5-La_2O_3-Ag (4 wt.%).

After modification with silver, broadening and shift of the peak in the short-wavelength region up to 449 cm^{-1} in composite CeO_2-Ag and up to 439-445 cm^{-1} in composite La_2O_3-Ag was observed (fig.3 (2-a,b)).

Biological activity of silver-doped nanocomposites

Biocompatibility, toxicity and ability to penetrate cells are the main criteria that determine the effectiveness of nanoparticles and nanocomposites in medicine. The analysis of lanthanum-cerium (La_2O_3-CeO_2), lanthanum-silver and cerium-silver NCs cytotoxicity was carried out (fig.4). Lanthanum-cerium at all used concentrations does not significantly effect on BHK-21 cell viability. However, the addition of different argentum weight ratio to lanthanum and cerium oxides increases their toxic effect on cells.

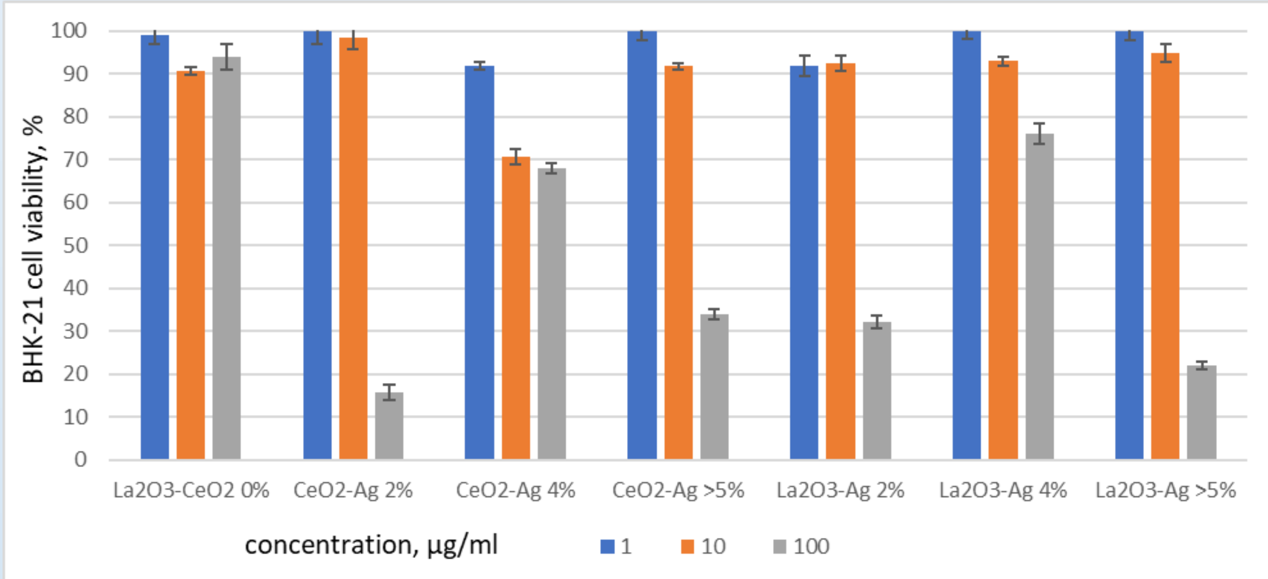

Figure 4. Effect of NCs on mitochondrial activity of the cells

Antiherpetic activity of silver containing NCs added at different stages of the herpes simplex virus life cycle was detected. The most direct way to suppress viruses is to inactivate them, and some of the nanostructures can interact with viruses, change their capsid protein structure and then dramatically reduce the virulence. It was found that depending on the weight ratio of argentum in NCs, their ability to effect on extracellular HSV-1 also changes. Exposure of the HSV-1 with lanthanum and cerium oxides doped with 2 and 5% argentum for 60 minutes completely blocked virus reproduction (fig.5).

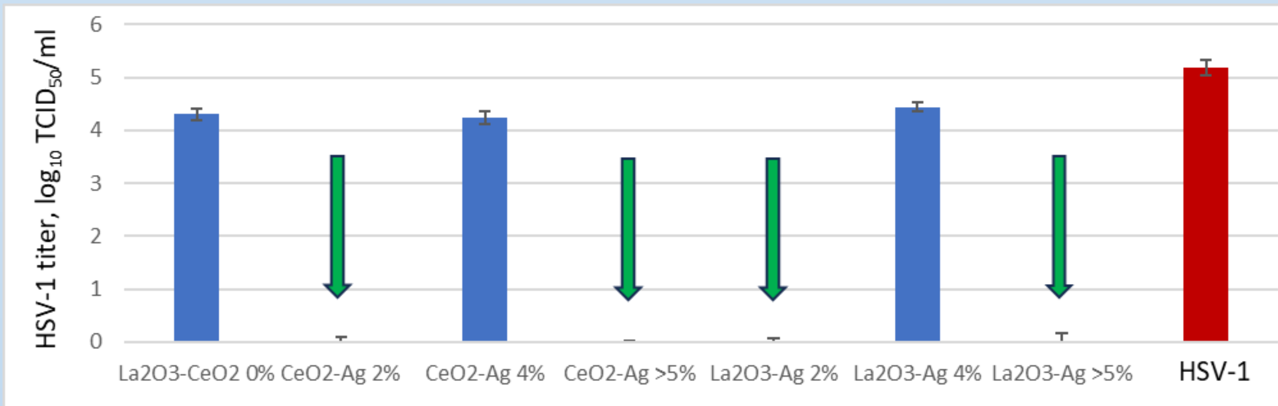

Figure 5. Virucidal activity of composites

Analysis of the antiherpetic effect of NCs added during HSV-1 adsorption and penetration stages showed that regardless of their structure and concentration, NCs are unable to block the early stages of herpes virus reproduction (the decrease of the virus infectious titer does not exceed 0.2 log_{10}).

In the case of virus entry into cells, destroying their replication or suppressing of virus budding and excrete it from host cells are an effective way to inhibit the virus. If NCs will be preventing the virus reproduction reduce the number of offspring viruses. the virulence will be reduced to an extraordinary degree. High antiherpetic efficiency of the lanthanum-cerium nanocomposite at the late stages of virus reproduction was established (fig.6). Thus, for the application of the La_2O_3-CeO_2 at a concentration of 10 µg/ml post infection of the cells, a complete blocking of the synthesis and formation of full-fledged and infectious offspring of HSV-1 was found.

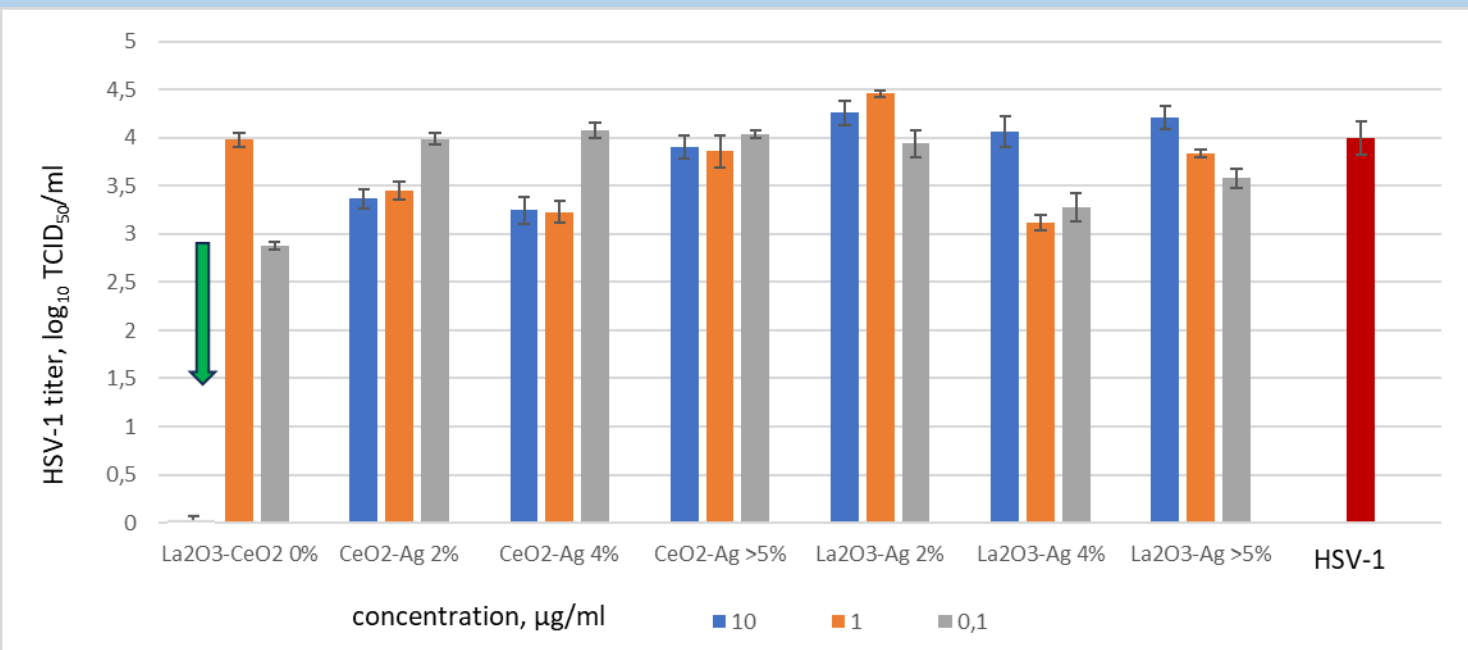

Figure 6. Effect of nanocomposites on HSV-1 infectious titer (post-entry treatment)

Conclusion

The obtained data indicate the promising use of lanthanum-cerium, cerium-silver and lanthanum-silver nanocomposites for the development of herpes infection control agents.