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Virucidal activity of polymer silver-containing films against enveloped viruses

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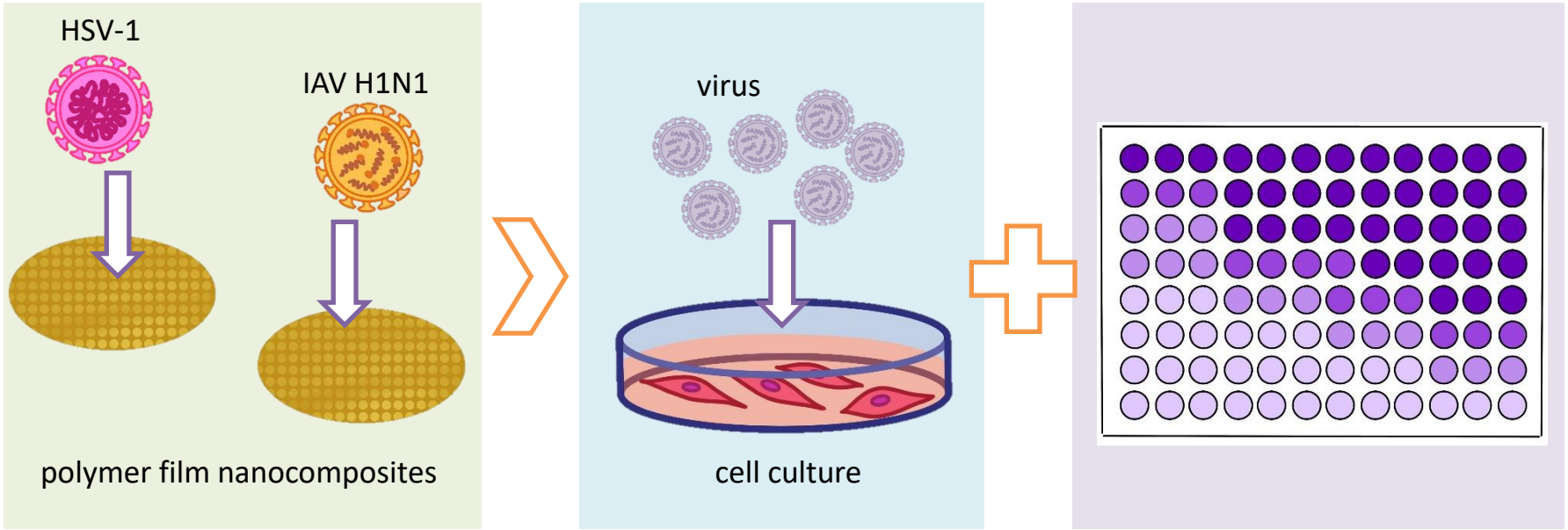
Graphical Abstract



Abstract:
The comprehensive approach to antivirals is needed to successfully fight viruses. Virucidal agents are important for reduction of the viral spread. Among nanoparticles, silver ones are able to directly interact with virions and in combination with right substances their effect can be enhanced. **The goal** of this work was to study the toxicity and virucidal activity of silver-containing films based on low molecular weight chitosan (LMWC) and sodium carboxymethylcellulose (Na-CMC) polyelectrolyte complexes against influenza A virus H1N1 (IAV) and herpes simplex virus 1 (HSV-1). We also compared the effect of silver-containing films (LMWC-Na-CMC-Ag) obtained by the laser ablation method and the green synthesis using the ginger extract (GE). **Methods.** The MTT-assay was used for assessment of cytotoxicity in MDCK and Vero cell lines. Virucidal activity was determined by the pre-incubation with the virus for 1 hour followed by determination of the infectious titer ($TCID_{50}$/ml). **Results**. Out of all the tested samples, only the ginger extract reduces cell viability by more than 50%. The virucidal effect of the extract manifested itself as a complete reduction of the infectious titer of both viruses. The LMWC-Na-CMC-Ag-GE showed the best virucidal activity with decrease in the infectious titer for 1.88 (IAV) and 3.79 (HSV-1) $\log_{10}$ $TCID_{50}$/ml compared to the virus control. However, LMWC-Na-CMC-Ag obtained by the laser ablation method was not effective. **Conclusion**. Thus, the polymer silver-containing film obtained by the green synthesis considering the lack of toxicity and virucidal effect against enveloped viruses is promising for further research.

Keywords: green synthesis; chitosan films; silver nanoparticles; influenza virus; herpes simplex virus.

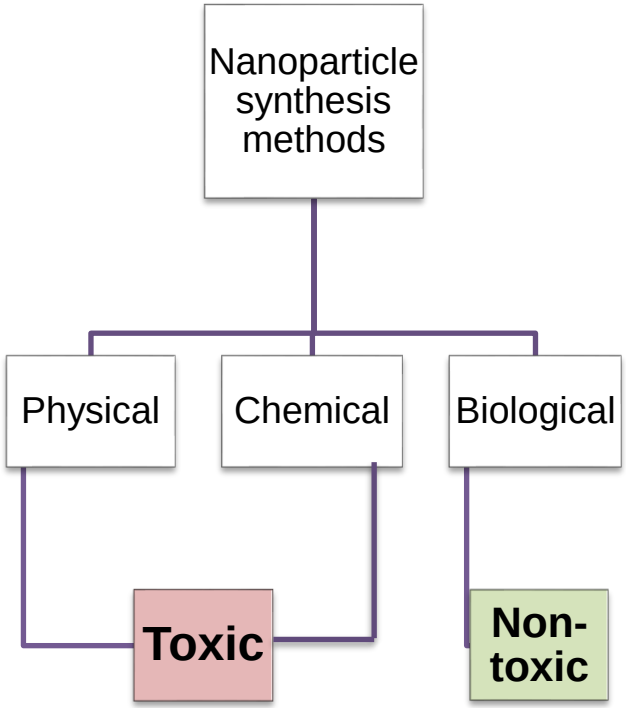
Introduction

In the list of causative agents of acute respiratory diseases, **influenza viruses** have a permanent place, but the problem of the efficient drugs lack still remains unsolved, in particular due to the peculiarities of RNA-viruses.

Herpes simplex viruses (both type 1 and type 2) are known for lifelong infections with reactivations due to the neuron infection, not to mention the high prevalence within the human population. Today, numerous anti-herpetic drugs are known, but new approaches and improvements to this field are always welcome.

The approach to solving the problem of the efficient antivirals lack should be comprehensive. We need not only etiotropic antiviral agents, but also virucidal, to reduce the spread of viruses.

Introduction



- Silver nanoparticles have shown the ability to directly interact with virions, and special attention is paid to nanocomposites that improve the properties of this metal.
- Chitosan is the second abundant polysaccharide in nature after cellulose and is widely used for the antimicrobial hydrogels and films. Since it extremely expands the surface area by allowing a further effective interaction of nanoparticles with pathogens. Furthermore, there are studies that prove the antibacterial efficiency of combining silver nanoparticles and chitosan.
- But nanomaterial industry places a massive burden on the environment by formation of hazardous byproducts and high power consumption. Green synthetic approaches offer eco-friendly, sustainable, nature-derived alternative production methods, thus attenuating the ecological footprint of the nanomaterial industry.

The goal of this research was to study the toxicity and virucidal activity of silver-containing film materials based on low molecular weight chitosan and sodium carboxymethylcellulose complexes against influenza A virus and herpes simplex virus type 1 as well as to compare the effect of film nanocomposites obtained by the laser ablation method and the green synthesis.

Materials

Cell cultures

- MDCK
- Vero

Viruses

- Influenza A (H1N1) virus strain A/FM/1/47 (IAV)
- Herpes simplex virus 1 strain US (HSV-1)

Studied samples

1. Ginger extract (GE), w=8.2%, ethanol-glycerol based

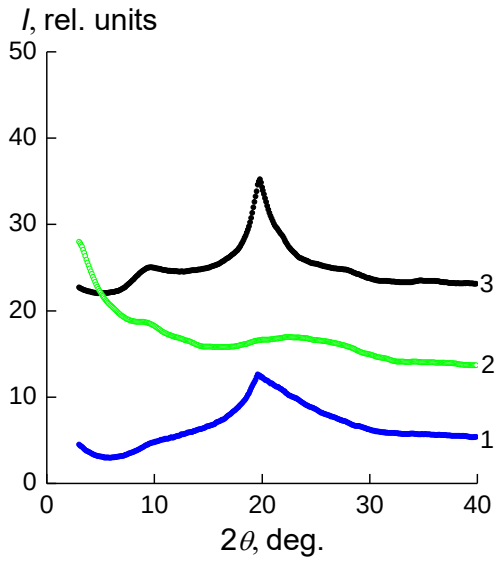
- **Polymer film nanocomposites**

2. LMWC (low molecular weight chitosan)
3. Na-CMC (sodium carboxymethylcellulose)
4. LMWC-Na-CMC
5. LMWC-Na-CMC-GT

- **Silver-containing film nanocomposites**

6. LMWC-Na-CMC-Ag (laser ablation method)
7. LMWC-Na-CMC-Ag-GE (green synthesis using the ginger extract)

Structure of the studied film nanocomposites



Wide-angle X-ray diffractograms of Na-CMC (1), LMWC (3) and LMWC-Na-CMC complex (2)

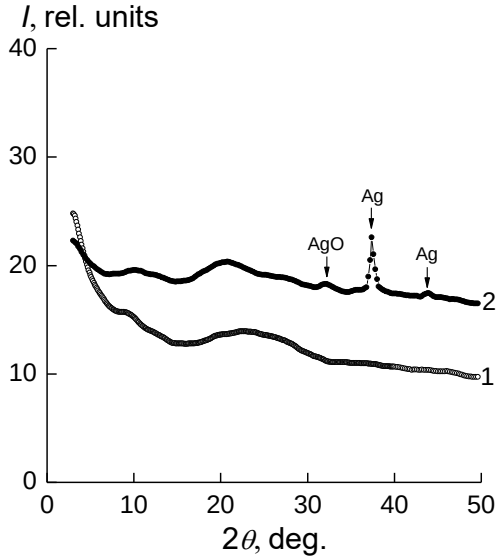
Analysis of wide-angle X-ray diffractograms showed that Na-CMC has an amorphous structure of both main macrochains and side branches. This is indicated by the manifestation of two asymmetric diffraction maxima of the diffuse type (amorphous haloes) of different intensities (curve 1), at $2\vartheta_m$ ~ 19.6° (the main maximum) and at $2\vartheta_m$ ~ 9.9° (the secondary one).

LMWC is characterized by an amorphous-crystalline structure. This is indicated by the presence of two diffraction maxima at $2\vartheta_m$ ~ 9.2° and 20.0° against the background of an imaginary amorphous halo at $2\vartheta_m$ ~ 20.0° on the diffractogram (curve 3).

A new amorphous structure is formed when mixing anionic and cationic polyelectrolytes Na-CMC and LMWC (curve 2). This is indicated by the diffraction maximum of the amorphous type at $2\vartheta_m$ ~ 9.5°, as well as an amorphous halo at $2\vartheta_m$ ~ 23.6°.

Structure of the silver-containing nanocomposite LMWC-Na-CMC-Ag-GE

The reduction of Ag^+ ions with the help of ginger extract to metallic silver is evidenced by the presence on the diffractogram of two low-intensity maxima at $2\vartheta_m$ ~ 38° and 44°, which correspond to the crystallographic planes of the face-centered cubic lattice of silver and confirm the presence of metallic silver in the polymer system (curve 2).
Also, a maximum at $2\vartheta_m$ ~ 32.1° characterizes the structure of AgO.

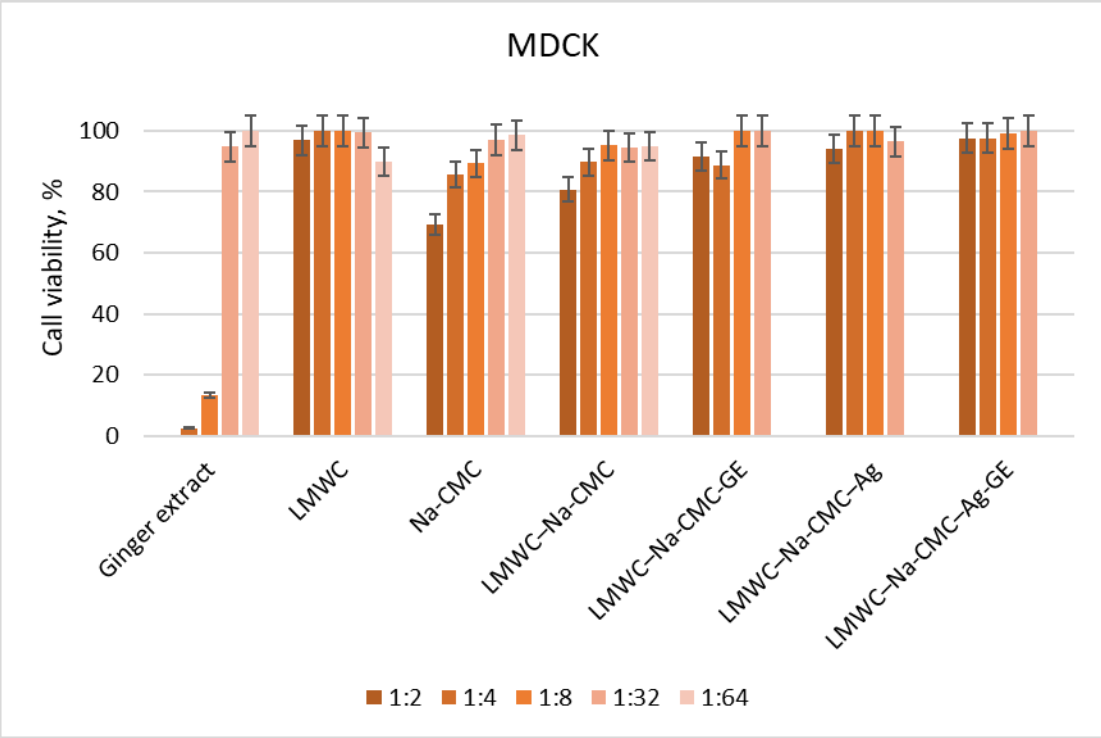


Wide-angle X-ray diffractograms of LMWC-Na-CMC (1) and silver-containing complex LMWC-Na-CMC-Ag-GE (2)

Methods

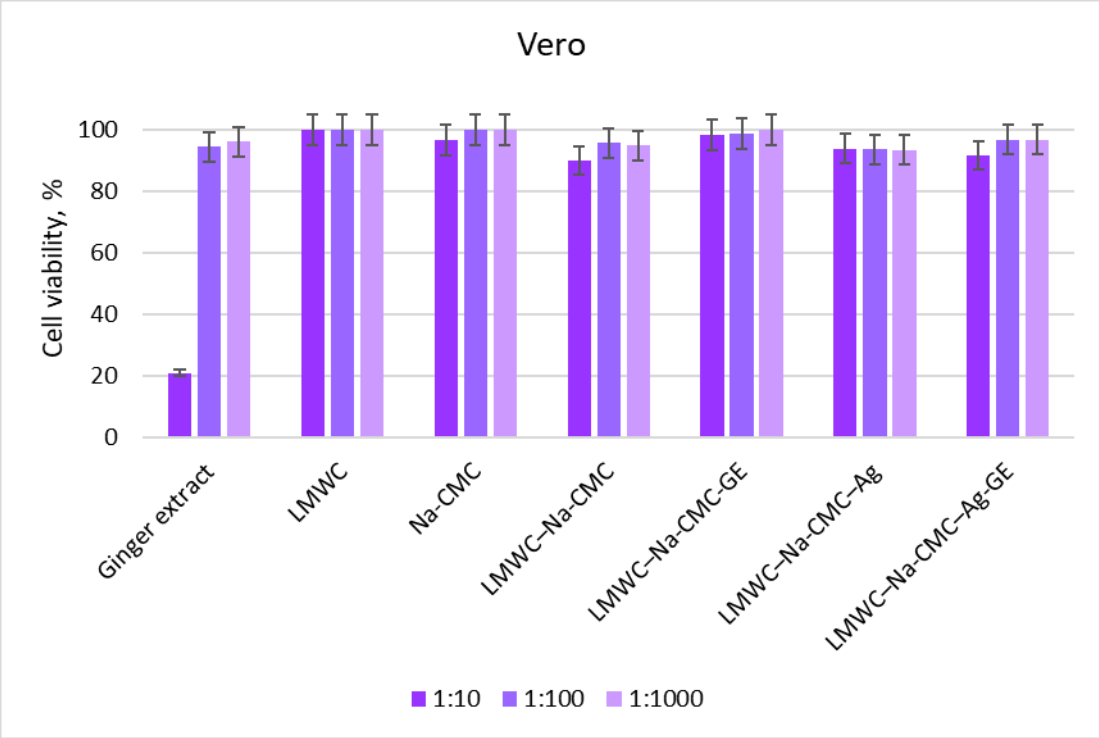
- The cytotoxicity was determined using the MTT assay.
- Virucidal activity was determined by the pre-incubation of the polymer films with the virus for 1 hour at 37°C followed by determination of the infectious titer ($TCID_{50}$/ml).

Results and discussion: cytotoxicity



Among the studied samples only the ginger extract reduces cell viability by more than 50% in MDCK cells. Therefore, CC_{50} index for the extract in MDCK cells is 1:19.02±0.95 in dilution or 0.43% in mass fraction of dry matter.

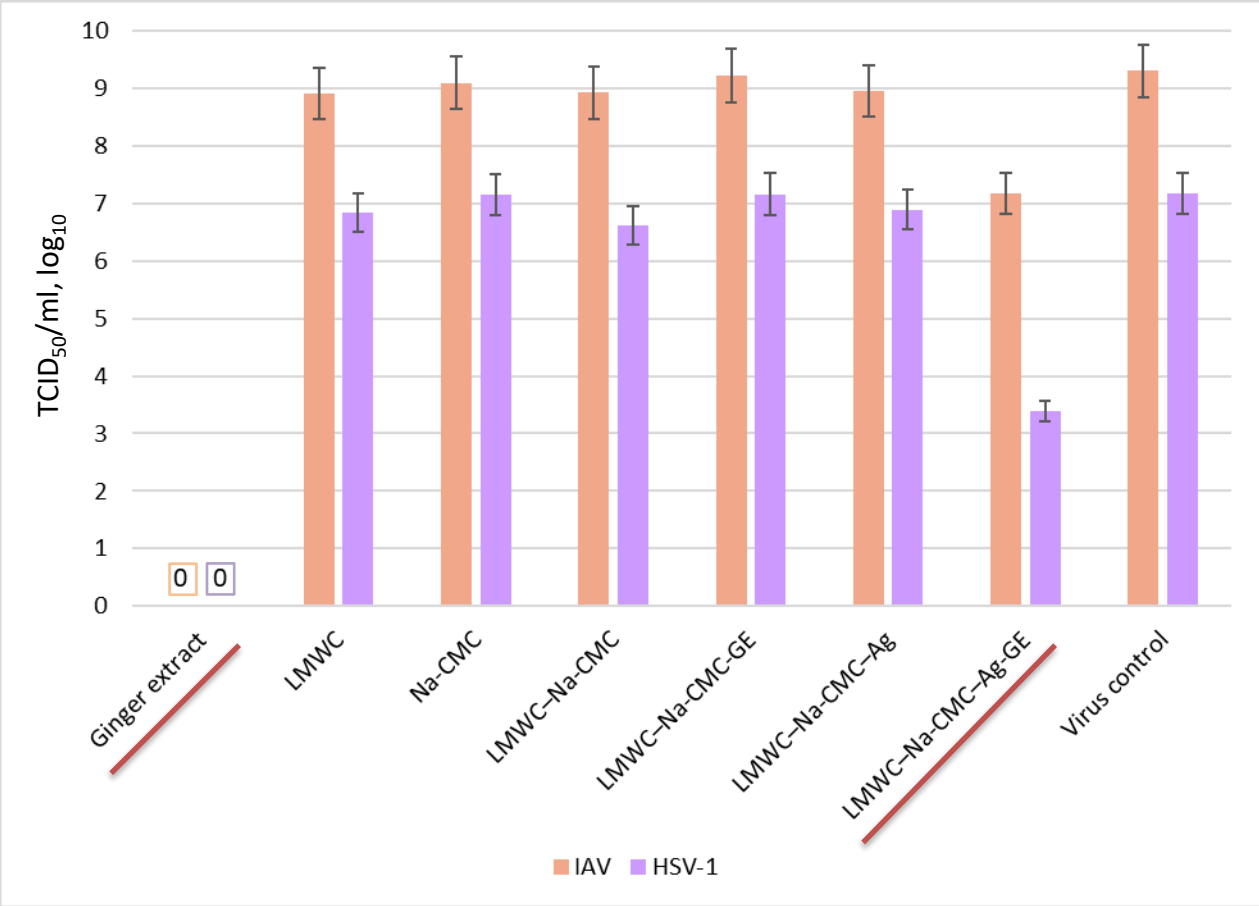
Results and discussion: cytotoxicity



Similar to the previous results, only the ginger extract reduces cell viability by more than 50% in Vero cells. Its CC_{50} index in Vero is 1:46.24±2.31 in dilution or 0.17% in mass fraction of dry matter. Thus, the polymer film nanocomposites are non-toxic for the MDCK and Vero cell cultures.

Results and discussion: virucidal activity

Among tested nanocomposites ginger extract and LMWC-Na-CMC-Ag-GE demonstrated distinct virucidal activity.



Results and discussion

- The virucidal effect of the ginger extract manifested itself as a complete reduction of the infectious titer of both IAV and HSV-1.
- The LMWC-Na-CMC-Ag-GE showed the best virucidal activity among polymer films with decrease in the infectious titer for 1.88 (IAV) and 3.79 (HSV-1) $\log_{10}$ $TCID_{50}$/ml compared to the virus control.
- The silver-containing nanocomposite LMWC-Na-CMC-Ag obtained by the laser ablation method was not effective in comparison with LMWC-Na-CMC-Ag-GE obtained by the green synthesis method.

Conclusions

The polymer silver-containing film nanocomposite obtained by the green synthesis LMWC-Na-CMC-Ag-GE is promising for further research considering the lack of toxicity and the virucidal effect against both enveloped DNA and RNA viruses.

Therefore, the green synthesis makes it possible to obtain effective silver-containing film composites with virucidal activity against enveloped viruses. Accordingly, this may be important for many viruses that have airborne and fecal-oral routes of transmission. And usage of nature-derived components is definitely a great approach to the production of nanomaterials.

Acknowledgements

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