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Research Article

Antifungal properties of SiO_2/hydroxypropyl cellulose hybrid materials doped with zinc ions against *Candida albicans* 74

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Abstract

This study presents an investigation of antifungal properties of hybrid materials based on silica, hydroxypropyl cellulose and $ZnSO_4.7H_2O$. The hybrid materials were synthesized by sol-gel technique and as a source of silica was used tetraethylortho silicate (TEOS). The quantity of organic substance was 5 wt. % and the zinc concentration varied from 0.5 to 5 wt. %. The strain that was used for determination of potential antifungal activity of materials was *Candida albicans* 74. The first experiment was focused on counting the survived cells after contact of fungi and materials with different zinc concentrations. The results show that the zinc amount and the cultivation time influence the number of colonies. The second method was based on measuring the free zone of fugal growth formed around well with materials. It was examined that the zone size depends on the amount of zinc ions in the materials. Practical applications: Metals such as silver, gold, copper and zinc are well known for their antibacterial activities and can be stabilized as nanoparticles by including in different polymers or hybrid materials with organic or inorganic nature. These materials can be used as coating layers on prostheses or catheters used in medical practice. They could be modified and used as facemasks, tissue paper, etc. in health centers. The broad spectrum antibacterial and antifungal activities of hybrid materials would be useful in preventing environmental contaminations on surfaces such as door handles, light switches and others in healthcare environments.

Keywords: Cellulose derivates, SiO2, Zinc ions, *Candida albicans* 74

Abbreviations:

HPC - Hydroxypropyl cellulose
NPs - Nanoparticles
TEOS - Tetraethylortho silicate

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Introduction

Nanotechnology offers the possibilities of great advancements in a variety of industries by manipulating materials on the atomic or molecular level and thus obtain novel characteristics and functions of smaller constructed materials (Meyer et al. 2002). The introduction of novel powerful antimicrobial agents is of great importance for the control of pathogenic bacteria (Tayel et al. 2011). Recent studies have demonstrated antimicrobial activities of various materials, containing nanoparticles (NPs) as silver (Angelova et al. 2012), copper (Cioffi et al. 2005), titanium dioxide (Kwak et al. 2001). The mechanism of the inhibitory effect of Zn nanoparticles on microorganisms is not fully understood. Preliminary studies show that the antibacterial activity of ZnO NPs might be related to the formation of free radicals on the surface of the NPs, and the damage to the lipids in bacterial cell membrane by free radicals, which consequently lead to the leakage and breakdown of bacterial cell membrane (Brayner et al. 2006; Reddy et al. 2007). The effect and mode of action of ZnO NPs on the growth of fungi is studied by He et al. 2011. They suggest that ZnO NPs may affect cell functions and finally cause the increase of nucleic acid contents. The increase of nucleic acid may due to stress response of *fungal hyphae*. The increase of carbohydrates may be due to the self-protecting mechanism against the ZnO NPs (Alvarez-Peral et al. 2002). In this study we examined the antimicrobial effect of SiO2 based hybrid nanomaterials with cellulose ethers and zinc ions against *Candida albicans* 74

Materials and Methods

Materials. Hydroxypropyl cellulose (HPC), tetraethylorthosilicate (TEOS) and zinc sulfate ($ZnSO_4.7H_2O$) were purchased from Sigma-Aldrich Chemie GmbH and sulfuric acid was from Merck, (Darmstadt, Germany). All chemicals were used as received without any further purification. The strain *Candida albicans* 74 was obtained from the culture collection of Bulgarian National Bank of Industrial Microorganisms and Cell Cultures and was maintained on nutrient YM medium, containing Yeast extract 3 g, Malt extract 3 g, Peptone 5 g, Glucose 10 g, Agar 15 g, d. H_2O 1 dm^3. The strain was cultivated at 30^0C.

Methods.

- Synthesis of HPC/SiO_2 hybrid materials doped with zinc ions. Hybrid materials were prepared by sol-gel technology. It is a quite used technique and needs simple equipment. Materials contained TEOS and 5 wt.% HPC and zinc with concentration from 0 wt % to 5 wt %. The procedure for hybrid preparation has been described in details elsewhere (Rangelova et al. 2014).

- Method based on quantitative determination of survived cells after contact with materials. The main point of this experiment is studying the ability of materials to influence the growth rate of culture after different period of incubation. The scheme of procedure is shown on Fig.1.

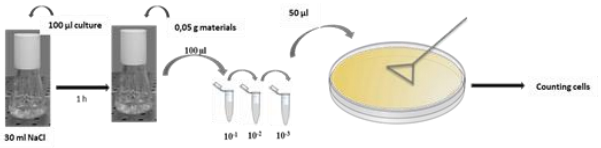

Figure 1. Scheme of quantitative determination of survived cells after contact with materials.

To each flask containing 30 ml saline solution was added 100 µl culture and after one hour 0.05 g of materials in form of powder. The process was performed on a rotary shaker (220 rpm) at 30°C. Samples were taken after 3, 5 and 24 h, they were diluted 10^{-1}, 10^{-2} and 10^{-3}, and 50 µl of each dilution was spread on agar surface. The following step is incubation of petri dishes and counting the number of colonies from each dilution on the plate. To evaluate the antifungal effect of investigated hybrid materials, the percentage of cell reduction was calculated as described by Riverro et al. 2011.

- Method based on diffusion of materials into agar and formation of inhibition zones. The method consists in formation of a clear zone (restricted microbial growth) around hybrids. Petri dishes with agar medium were inoculated with 100 µl fresh culture of *C. albicans* 74. After 30 min of

suspension penetration were made wells into agar, where 100 mg of each sample were placed. The inoculated plates were stored at 30°C with the agar on the upper surface. The size of the inhibition zones was monitored and measured with ruler after 24 h of strain incubation (Fig. 2).

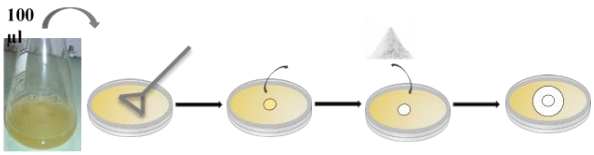

Figure 2. Scheme of diffusion test by formation of inhibition zones.

Results and Discussion

In our previous investigations with the same hybrid materials, we found that they show well pronounced antifungal effect against *Aspergillus niger* 371 and *Penicillium chrysogenum* 2303. To increase the information about the antimicrobial behavior of tested hybrids we direct our attention to fungi strain with clinical importance *Candida albicans* 74.

The first part of experiments was focused on the counting of survived cells after exposure to the hybrid materials. On the third hour of incubation it was observed, that the number of cells were reduced with increasing of zinc concentration in the materials (Fig. 3).

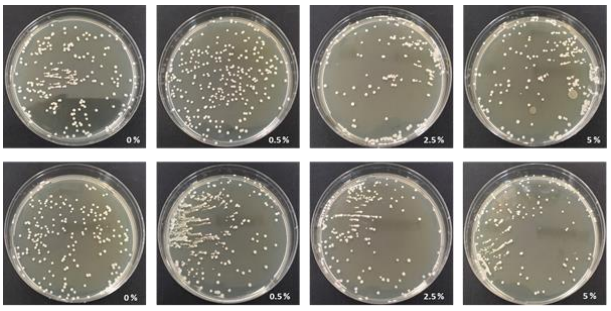

Figure 3. Growth inhibiting effect of SiO_2/HPC hybrid materials with different $ZnSO_4.7H_2O$ concentrations against *C. albicans* 74 after 3 hour exposure to the hybrids (first row) and 5 hours (second row).

The same tendency was examined after long time of contact between culture and materials. The number of cells decreased proportional to the increase of zinc concentration. It was examined that the time of cultivation didn't have any significance for the number of survived cells. The highest percent of reduction was calculated after 24 h cultivation by SiO_2/HPC/$ZnSO_4.7H_2O$ hybrids with 5 wt.%. In another our paper we examined that the same hybrid material but with 2.5 wt.% $AgNO_3$ showed almost the same inhibition effect of growth of *Candida albicans* 74 (Georgieva et al. 2015). In presence of 2.5 wt.% zinc ions the materials reduced the fungal growth with 75.8%. The second experiment considers the question for possibility of materials to form zones free of fungi growth. Using the agar well diffusion method, the hybrid materials supplied with different zinc concentrations showed a significant effect of fungi inhibition. After 24 h cultivation it was observed that hybrid materials doped with zinc proved clear zone of inhibition around the materials (Table 1).

Table 1. Antifungal test results of *C. albicans* 74 after 24 h cultivation.

Zn [wt. %]	SiO_2/HPC/$ZnSO_4.7H_2O$	
	CFU/ml	Reduction of cells [%]
0	$5.8x10^5$	-
0.5	$2.3x10^5$	60.4%
2.5	$1.4x10^5$	75.8%
5	$1x10^5$	82.7%

The sample with the highest 5 wt.% zinc concentration showed the clearest and biggest zone of inhibition – 11.75 mm. The potential of materials to prove antimicrobial effect decrease with small zinc concentrations. The results reveal that 2.5 wt.% Zn formed an inhibitory zone of 7.25 mm. The lowest concentration – 0.5 wt.% - can't influence the strain growth and it was impossible to measure any free zones (Fig. 4). It was established that size zone depended on the zinc concentration.

Figure 4. Inhibition zones of *C. albicans* 74 around the SiO_2/HPC hybrid materials with different $ZnSO_4.7H_2O$ concentrations.

Conclusions

The investigated SiO_2/HPC hybrid materials doped with zinc ions demonstrated pronounced antimicrobial activity against *Candida albicans* 74. It was demonstrated that increasing zinc concentrations up to 5wt.% led to larger inhibition zones around the materials and showed 82.7% reduction of cells. The investigated materials reveal good antibacterial and antimicrobial effect but further experiments are needed to confirm their non-toxicity activity against mammalian cells and they could be a promising candidate for future biomedical application. But tools and techniques are needed to be investigated for differentiation between particle-induced toxicity and dissolved Zn^{2+} effects.

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