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Amorphous hydrogenated carbon doped with copper as antifungal protective coating

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Abstract

We report on the fungistatic properties of amorphous hydrogenated carbon doped with copper. It is clearly demonstrated that copper forms clusters with sizes of some nanometers. These clusters are distributed homogeneously over an amorphous carbon matrix. Doped films, in contrast with undoped ones, inhibit fungi growth at the film's surface. This observed phenomenon is promising for the practical application of amorphous carbon films as a protective media against biodeterioration.

Keywords: A. Amorphous carbon; B. Plasma sputtering; C. Transmission electron microscopy (TEM); Optical microscopy; D. Biocompatibility

1. Introduction

The unique nature of novel allotropic carbon modifications and related phases, including diamond-like (DLC) and amorphous carbons [1], accounts for the possible interest in their effect on living matter. Our goal is to demonstrate a new biological property of hydrogenated amorphous carbon (a-C:H)-based composites – to be an active barrier against a biological attack of microorganisms. These are responsible for undesirable biodeterioration, which is known to be a scourge to up-to-date high technology products operating in closed spaces of satellites, aircraft, submarines etc. DLC and a-C:H films are known to be good protectors against environmental pollutants [2] and atmospheric waste as well. It was shown recently that modification of a-C:H films with nanosize copper clusters (a-C:H:Cu) by ion co-sputtering of copper and graphite targets allows producing films with entirely new properties [3]. In the present paper we show that among these properties is a strong fungistatic (i.e. inhibiting the fungal growth) effect of these films. We demonstrate that copper nanosize clusters embedded in a-C:H act as a toxic agent for microorganisms. A mechanism of copper liberation from the a-C:H matrix is discussed.

Our goal is to demonstrate quite a challenge of high technology of creation of a-C:H-based composites to create substances operating as active barriers against biological attack of microorganisms which normally play an important role in natural environments in recycling the elements of the Earth's crust. These microorganisms, however, are able to cause biodeterioration. Among other problems necessitating creation of a substance having protective abilities, is the possible toxic damage to higher animals and human beings. We selected a carbon-based technology of ion sputtering of a carbon-based target as a tool to produce a selective protective coating. Namely, we used a-C:H films, unique coatings, which can be applied to many surfaces – plastics, ceramics, glass, etc. [1]. Copper trapped into the a-C:H matrix forms nanosize clusters which modify integral characteristics of an a-C:H film [3]. In the present work we modify the a-C:H matrix so as to impart to a-C:H, an additional property – fungistatic action. This modification is of fundamental interest (providing a possibility to study the interaction between fungi and nanoclusters) and will extend the area of application of a-C:H. Under normal conditions (temperature 20°C, humidity 70–98%, pH=6.5), copper clusters are inert

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because of the properties of the a-C:H matrix. We report here a fungistatic action of a-C:H films containing copper nanoclusters (a-C:H:Cu) against a microbiological attack. Copper is known to exhibit a wide spectrum of toxic actions against diverse microbiological objects, including fungi [4].

2. Methods

2.1. Method for a-C:H film growth

The a-C:H films were grown on various substrates by ion co-sputtering of graphite and copper targets in argon–hydrogen (80% Ar and 20% H_2) plasma with a DC magnetron. Glass, Lavsan polymer and polished KBr plates were used as substrates, depending on specific experimental requirements. All the films had good adhesion. The substrate temperature, gas pressure in the growth cell, and average magnetron power were 500 K, 10 mTorr and 0.4–0.5 kW, respectively. The magnetron voltage was 350–450 V. The copper concentration in the growing film, varied within the range 0–12 at. %, was controlled by changing the relative areas of graphite and copper targets being calibrated by secondary ion mass spectrometry (SIMS) measurements. The films thickness was within the 100–150 nm range. This method is described in more detail in [7].

2.2. Method for TEM

For TEM image of copper nanoclusters in free-standing, approximately 100 nm thick films were prepared by dissolving KBr substrates in water. The study was performed with a Philips 400 EM transmission electron microscope operating at 100 keV. The size variation of copper-born nanoclusters was analysed from TEM micrographs directly and the resulting size-distribution function is shown in Fig. 1a,b, where clusters of a 2.9 nm average diameter, distributed in the a-C:H matrix, can be seen. Analysis of the distribution demonstrates its Gaussian character conforming to the fluctuation theory of phase nucleation [7].

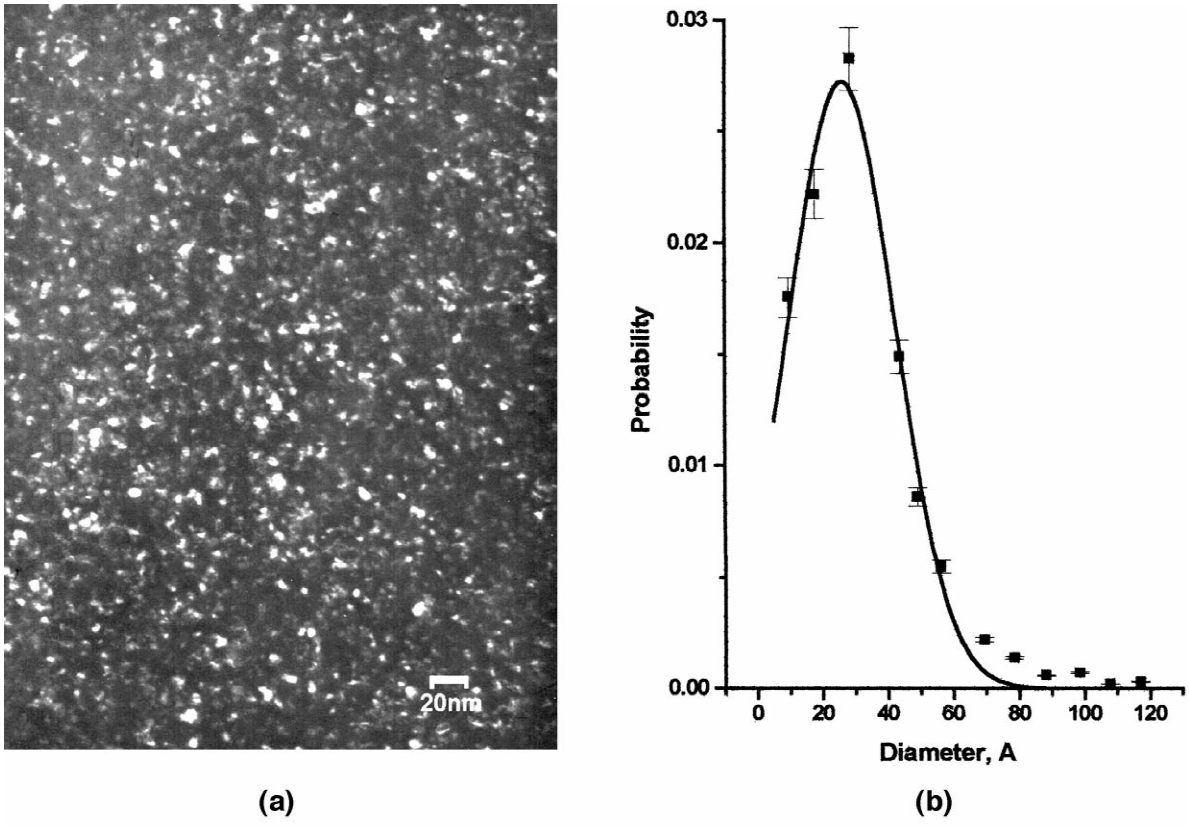
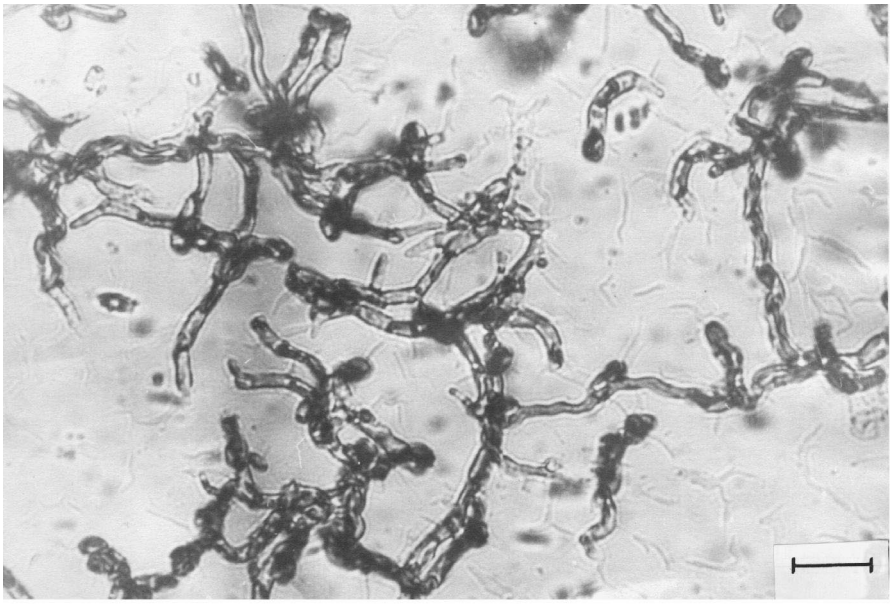


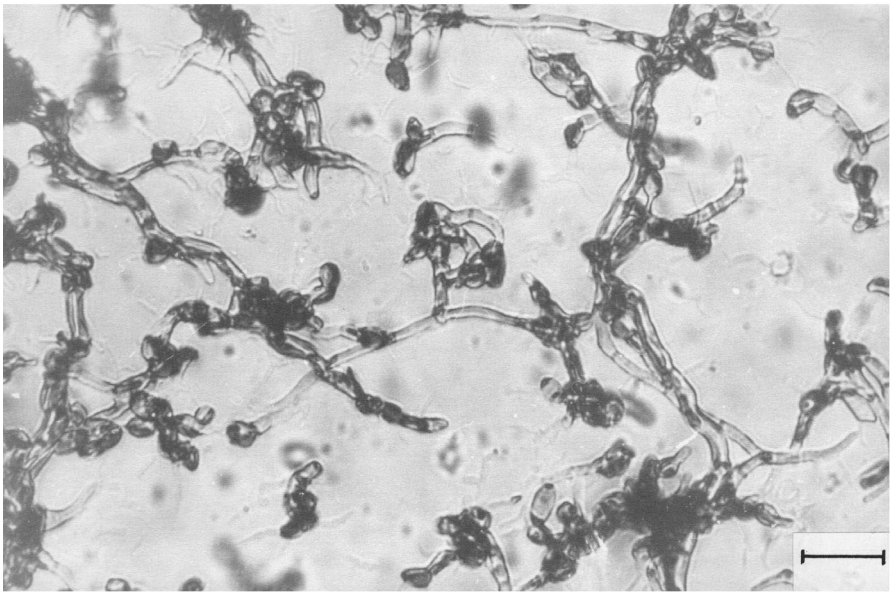
Fig. 1. Electron micrograph of copper nanoclusters in a-C:H:Cu film and their size-distribution function. (a) Dark-field TEM image of copper nanoclusters in an a-C:H:Cu film, with a total copper concentration of about 9 at. %. Bar is 40 nm. (b) Copper nanocluster size-distribution function as derived from analysis of the TEM image in Fig. 1a. The black squares are experimental data, and the full curve is a result of fitting with a Gaussian distribution function.

2.3. Estimation of biodeterioration of materials

To demonstrate the fungistatic action of a-C:H:Cu films, we used 5 species of fungi belonging to *Ascomycotina* and *Deuteromycotina*. Among them there are typical destructing agents of synthetic polymers: *Aspergillus niger* v.Thiegh, *Chaetomium globosum* Kunze, *Cladosporium cladosporioides* (Fres) de Vrien, and also rock-dwelling fungi, namely strains, which have been isolated [6] from marble: *Epicoccum nigrum* Link ex Wallroth and *Pestalotia heteromorpha* Thuem. The morphogenesis, growth rate and distribution of the fungi over the surface of the material were used as a guideline in characterising the fungi–substrate interaction. Glass and Lavsan polymer



(a)



(b)

Fig. 2. Photomicrographs showing the status of *C. cladosporioides* fungi after 21 days of cultivation on (a) a pure Lavsan polymer; (b) a Lavsan covered with films: a-C:H; and (c) an a-C:H:Cu (9 at. %). The scale bar is 15 μm.

(polyethylene terephthalate), both covered and uncovered with an a-C:H film, were exploited as substrates.

Samples of materials were inoculated with fungi spores (10^6 cm^{-3}, 4 h old) in distilled water (pH=6.8), and exposed in closed chambers for 21 days at T=27°C and 100% humidity. The reference group was exposed under similar conditions.

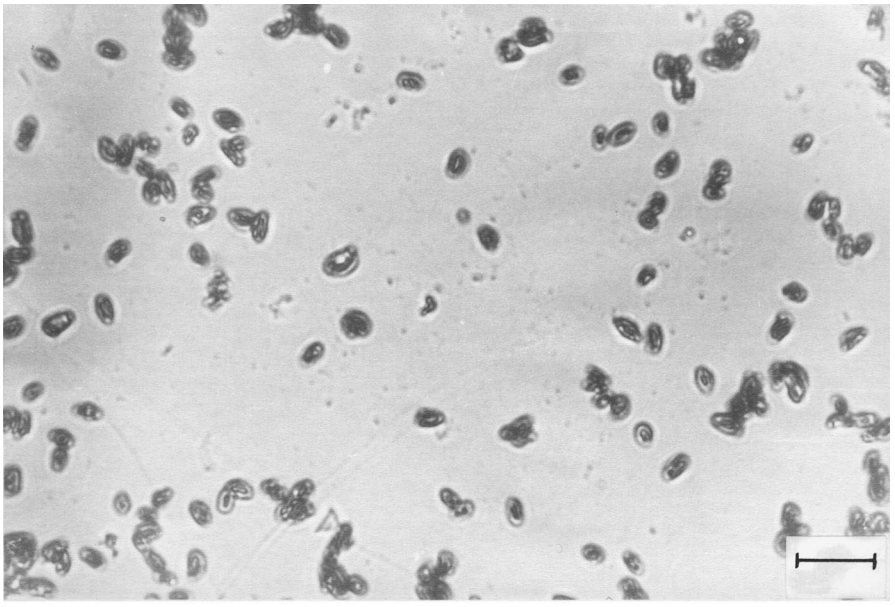
The degree of biodeterioration of samples has been estimated on the basis of fungi growth. Growth monitoring has been carried out by means of a SMP-6 OPTON microscope, with subsequent image analysis of representative vision fields 0.5×0.5 mm (10 fields from every samples). Quantitative estimation of fungal growth, so called coefficients of biodeterioration (*K*), have been taken as a percent ratio between area occupied with fungi and the whole area of the analysed field.

3. Results and discussion

The TEM image of an a-C:H:Cu film (9 at. % Cu), presented in Fig. 1a, shows a large number of copper nanoclusters incorporated in the a-C:H matrix. A direct computer analysis of the image produces the size-distribution function of copper nanoclusters is shown in Fig. 1b. Both Fig. 1a and Fig. 1b indicate a rather homogeneous distribution of the clusters throughout the a-C:H film.

A-C:H films were fabricated as described in Section 2.1. The protective action of a-C:H:Cu films against fungal attack is illustrated in Fig. 2. There is no fungal growth pathology on the a-C:H film: the cell size, shape and branching are typical of the *C. cladosporioides* species. It can be seen that undoped a-C:H films are not able to withstand the fungal growth on their surface. At the same time, films containing 9 at. % of copper totally prevent fungal growth. The technological modes of preparation of films exhibiting fungistatic properties are determined by such parameters as film thickness and copper concentration. The maximum protective effect is obtained at copper concentrations in a-C:H close to the percolation threshold determined in our previous work [3,7]. The choice of parameters of these coatings is governed by additional requirements, e.g., by aesthetic or construction properties of an object to be protected. Since we have shown in preliminary experiments that the purely a-C:H matrix does not act as a biocide (antiseptic or antifungal) coating, the mechanism of the fungistatic effect of a-C:H:Cu films is based on the toxicity of copper for fungi. Copper is known to be involved in fungal metabolism [5] when taken at a 0.1–5 mg l^{-1} concentration. At the same time, at relatively high concentrations, it is one of the most toxic elements for fungi (see Cu^{2+} values, Table 1), along with Ag and Hg, especially in the form of Cu^{2+}, $Cu(OH)^{+}$, $Cu(OH)_2$ (aq.) [4].

The possible mechanism of copper liberation from the matrix was studied in terms of the influence of fungal metabolites on the copper in a-C:H. Simulation experiments (5 series, in 5 replications) were made using citric acid which is commonly produced by many fungal species, for example, *A. niger*. Onto the surface of a-C:H and a-C:H:Cu film, coating the glass substrate was deposited dropwise (50 μl drops) with a citric acid solution (pH=3)



(c)

Fig. 2. (*continued*)

Table 1
Fungistatic action of a-C:H:Cu films deposited onto polymeric and glass substrates

Fungi species	Differential coefficient of biodeterioration, K^*[a]				MIC[b]
	glass	a-C:H:Cu (9 at. %) glass	Lavsan	a-C:H:Cu (9 at. %) on Lavsan	Cu^{2+}, mg l^{-1}
A. niger	5.5	0	17.3	0	50
Ch. globosum	3.1	0	18.1	0	50
C. cladosporioides	3.9	0	16.4	0	100
E. nigrum	4.7	0	15.7	0	100
P. heteromorpha	4.3	0	15.9	0	100

[a] $K^*=K_0-K_1$, where K_0, K_1 – coefficients of biodeterioration of materials at 1 and 21 days.
[b] MIC=minimal inhibiting concentration of Cu^{2+} ions, taken as $CuSO_4 \cdot 5H_2O$ in liquid Czapek medium, defined by method [8].

and a reference solution of distilled water (pH=6.8) for 15 min. The concentration of copper ions passed into solution was estimated by optical chemosensors [6].

As a result, the average share of copper washed out by citric solution from a-C:H:Cu (9 at. %) films was 50% of 9 at. %, in comparison with 0% for distilled water. So, the a-C:H:Cu film is a nanocomposite containing copper clusters which can release copper under the action of organic acids produced by fungi. When the amount of released copper exceeds the threshold value for a certain species, it acts as fungistatic agent which suppresses the growth of the fungi.

So the modified with copper a-C:H coating offers clear advantages over conventional a-C:H alternative, and that approach allows to solve important problems of protection against biodeterioration.

4. Conclusion

It is clearly demonstrated that produced by magnetron sputtering of graphite the protective coating is not able to withstand a fungal attack. However, modified with copper such coating offers clear advantages over conventional amorphous carbon alternative, and that approach allows to solve important problems of protection against biodeterioration. This result is exciting because it highlights the usefulness of the nanotechnology for developing materials with reliable fungistatic action.

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