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Effect of nanotubes on the electrical and mechanical properties of chitosan films

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Using the methods of X-ray diffraction and scanning electron microscopy, the structure of composite films based on chitosan and single-wall carbon tubes has been studied. It is shown that the introduction of carbon nanotubes leads to the ordering of the chitosan structure. Increase in concentration of nanotubes (from 0 to 3%) causes rise in the value of storage modulus from 3 to 4 GPa (DMA data), increase in electrical conductivity of samples (from 10^{-11} to 10^{2} S/m), and some changes in their dielectric permittivity (from 5.5. to 26 at an electrical field frequency of 1 kHz). Data on the ionic and electronic components of the conductivity of the composite film are presented.

Keywords: chitosan, electrical conductivity, nanotubes, dielectric constant, composite.

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Introduction

Recently the polymeric materials are actively used in cell technologies, regenerative medicine, as well as in production of medical devices for diagnostics and treatment of various diseases. Films, fibers and modular porous materials, used in biomedical technologies, should be not only biocompatible, but also have a set of characteristics, that provide their biometric properties and specific bioactivity. This is achieved by maximum approximation of structure, as well as strength, deformation and electrophysical properties of the material to characteristics of living organism tissues [1]. It was also shown, that electrical stimulation using conducting materials is an effective technology for cells behavior regulation [2].

Biocompatible polymers, such as polylactide, polyglycolide and their copolymers, as well as chitosan can be used as a base for biologically active composite materials. Most of the polymers have dielectric properties, but at the same time over the last years the conducting composite materials, containing such components, as carbon particles of various shape and size — carbon fibers, technical carbon [3], as well as carbon nanotubes, nanofibers, graphene [4], as fillers, have been developed. Conducting polymers, particularly polypyrrole [4–6], can be used as the conducting components.

Single-wall carbon nanotubes (SWCNT) are promising fillers for composite materials of various purpose, their introduction allows to regulate mechanical, electrical and thermal characteristics of material. SWCNT are used as drug carriers [7–9], for biosensors, matrices for cell technologies and tissue engineering [4,10,11]. Recently they are effectively used as fillers for conducting composite materials [4], structures for effective control of neuritic stem cells growth [11,12].

Beside reactions of cells, that are traditionally considered as electrosensitive, the electrostimulation effect on human dermal fibroblasts culture for efficiency improving of the skin and soft tissues regeneration is described [13].

Data, indicating, that constant electrical potential maintaining between external and internal skin layers is required for its homeostasis, and in case of skin damage the electrical signals coordinate the regeneration processes, also speak in favor of electrosensitivity of dermal fibroblasts [14].

Chitosan is one of the promising polymers for using as nanocomposites matrix for skin regeneration technologies. Being a derivative of chitin polysaccharide, it has a full range of biological properties, such as biocompatibility, bioactivity, bioresorbability, antibacterial, wound healing and hemostatic activity [15]. While materials based on chitosan have useful healing characteristics, they do not have a required level of electrical conductivity. At the same time, the electrical signals participate in cell communications, accompanying tissue regeneration. Therefore, the matrices for tissue engineering should have a certain level of electrical conductivity to improve their biocompatibility, stimulate the

cell processes, contribute to adhesion, proliferation and differentiation of cells [16,17].

Introduction of SWCNT in amount, not exceeding 10 wt.%, into the matrix based on chitosan allows to significantly increase material electrical conductivity, its strength and elastic characteristics [6,18]. At the same time, low filler content and its stable interaction with polymer allow to minimize its potential cytotoxicity.

The purpose of this work is a development of conducting composite films based on chitosan and SWCNT, as well as study of their structure, electrophysical, physical and mechanical and thermal properties.

1. Materials and methods

Composite films were made of SWCNT mixture with 4% chitosan solution in 2% acetic acid. Chitosan of Biolog Heppe GmbH, Germany, with molecular weight $M_m = (1.64-2.1) \cdot 10^5$, and deacetylation degree $DD = 92\%$ was used. Diameter of SWCNT, made by Carbon Chg, Russia, was 1.4 ± 0.3 nm, and length — $1-5\,\mu$m. Water dispersion, containing SWCNT, was processed with ultrasound using disperser IL10-0.63 for 15 min at frequency of 25 kHz and power of 630 W. Chitosan was introduced into SWCNT water dispersion in amount, providing polymer concentration in solution of 4.0 wt.% and the required ratio of chitosan/SWCNT.

Mixture of chitosan and SWCNT was mixed in water for 30 min until swelling and partial dissolution of chitosan. Then, the acetic acid was added to the resulting mixture; its concentration in solution was 2%. The solution was mixed for 180 min, then filtered and deaerated in vacuum chamber for 24 h at pressure of 10 kPa. SWCNT content was 0.1, 0.5, 1.0, 3.0 wt.% relating to chitosan. The films were poured by means of the solution extrusion through a slit die onto a glass substrate, dried at temperature of 50°C for 1 h. Then, the films on the glass substrate were deaerated in the vacuum chamber for 24 h at pressure of 10 kPa and then dried in air at room temperature for 24 h. For films transition from salt to the main form they were exposed in mixture of 10% water solution of NaOH and C_2H_5OH with components ratio of 1:1 for 10 min, and then they were washed with distilled water and dried in air. The thickness of the films ranged $30 \pm 5\,\mu$m.

Structure of nanocomposite chitosan films in the main form was studied using methods of scanning electron microscopy. Measurements were made using SUPRA-55VP device (Carl Zeiss, Germany).

Analysis of crystalline structure of chitosan and composite films was performed at DRON-3M diffractometer (IC „Bourevestnik“) in „reflection“ mode using CuK_α radiation. The recording was made in angles range $2\theta = 10-75°$ with pitch of 0.02° at exposition in each point of 1 s. The processing of the observed data and peaks identification were performed using software package DFWin and database ICDD PDF-2.

Conductivity was measured in isothermal conditions at 25°C using picoammeter Keithley 6487, both 2- and 4-electrode schemes were used. Dielectric spectra were observed at broad-band dielectric spectrometer Concept 22 made by Novocontrol Technologies with automatic high-resolution frequency analyzer ALPHA-ANB. Values of permittivity and losses were observed in frequency range from 1 Hz to 1 MHz, measuring signal amplitude was 1 V. Platinum electrodes with thickness of 10 nm were preliminary sputtered on samples. The samples were put between two parallel plate electrodes.

Mechanical properties were examined using Instron 5943 device, testing base — 10 mm, sample tensile speed — 10 mm/min. Before the tests the film samples of chitosan and composite films were exposed in exicator at relative air humidity of 66% for at least 24 h.

Temperature dependencies of elastic modulus (E') and tangent of angle of mechanical losses ($\mathrm{tg}\,\delta$) of the films of chitosan and composite films were examined through DMA method using DMA 242C/1/F unit (Netzsch, Germany). Measurements were performed during heating from 20 to 320°C with a rate of 5°C/min at loading frequency of $\nu = 1$ Hz, deformation amplitude of 0.1%, the sampling length was 10 mm. Temperature of the main relaxation transitions of the samples was defined as per temperature of curves maximums $\mathrm{tg}\,\delta$.

Thermal properties were examined using method of thermal gravitational analysis (TGA) using TG 209 F1 device made by NETZSCH (Germany). The tests were performed in temperature range from 30 to 800°C at heating rate of 10°C/min in argon medium. Sample weight was 2−3 mg. As a result, the remaining mass was defined at 800°C and thermal endurance indices (τ_5, τ_{10}) were calculated, values of which correspond to temperature of 5 and 10% loss of the samples weight.

2. Results and discussion

Study of composite materials structure was performed using X-ray diffraction and scanning electron microscopy. Diffractograms of films of chitosan and composite films with various SWCNT content are presented in Fig. 1. It is seen, that presence of two wide diffraction maximums at angles $2\theta = 10$ and 20° is characteristic for the film of chitosan in the main form, that is in good agreement with data, presented in the work [19]. SWCNT introduction into chitosan matrix results in rise of chitosan maximum intensity at angle $2\theta = 15°$, indicating the increase of chitosan crystalline regions ordering, particularly the increase of straightened chains share, decrease of distance between them.

Presence of carbon reflex [002] at angle $2\theta = 26.5°$, rise of its intensity indicate on presence of SWCNT in composite films, their concentration increase. This is confirmed with the results of SEM studies, presented in Fig. 2.

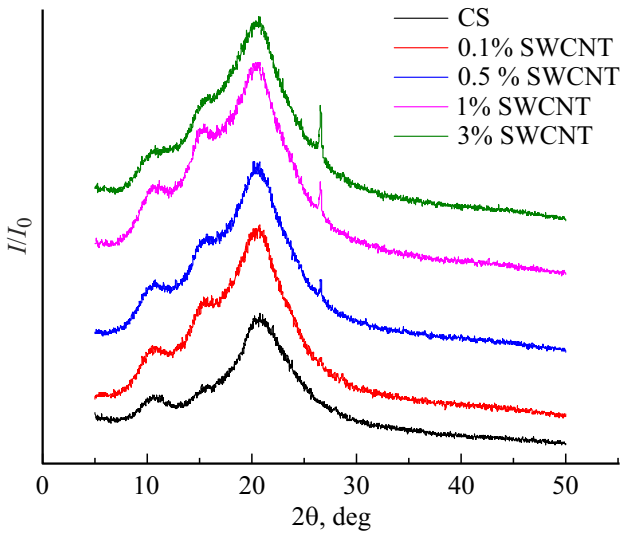

Figure 1. X-ray diffractograms of a film of chitosan (lower curve) and composite films with various SWCNT content (other curves).

Microphotographs of shears in liquid nitrogen of chitosan film and composite films, containing SWCNT, presented in Fig. 2, indicate that structure of chitosan film (Fig. 2, *a, b*) is characterized with presence of layered elements, that is agreed with data, presented in the works [20,21]. Addition of 0.5 wt.% SWCNT (Fig. 2, *c, d*) results in more dense, homogeneous film structure. This is confirmed with the results of X-ray structural analysis, presence of chitosan diffraction maximum at angle $2\theta = 15^\circ$ (Fig. 1).

If SWCNT content is increased to 1.0 wt.%, the individual carbon nanotubes are visible on shear (Fig. 2, *e, f*). Quantity of nanotubes on shear surface increases with addition of 3% SWCNT (Fig. 2, *g, h*). Saturation of chitosan matrix with carbon nanotubes results in formation of SWCNT grid, their location in a film plane, as well as normal to the plane of chitosan matrix, that is clearly seen in Fig. 2, h. Such filler structure should contribute to increase of composite material conductivity.

Dependence of specific electrical conductivity of chitosan films on SWCNT content is presented in Fig. 3.

As per the dependence, presented in Fig. 3, with SWCNT content increase from 0.5 to 1.0 wt.% the conductivity of the composite film increases from 10^{-7} to 10^{-2} S/m. Sharp increase of material conductivity at small change of filler content indicates on formation of conducting cluster, consisting of SWCNT.

Increase of filler content to 3 wt.% results in further rise of conductivity, but its rise rate slightly decreases. As seen from SEM data (Fig. 2, *g, h*), at such SWCNT content the distribution by volume of the composite film for anisometric particles of filler changes. Tubes, located not only in the film plane, but perpendicular to it are visible. This can explain the reduction of conductivity rise, measurement of which was performed in the film plane.

In the work [22] it was shown, that electrical conductivity of the non-filled chitosan film is 10^{-11} S/m. Skin conductivity is in a range from 10^{-5} to 10^{-2} S/m [23]. Thus, the composite films based on chitosan, containing 0.5–1.0 wt.% SWCNT, have electrical conductivity, close to the values of biological tissues, particularly human skin. This will allow to use the observed materials in tissue engineering.

Biological compatibility of polymeric matrix significantly depends on its electrophysical properties [24]. Beside electrical conductivity, the important characteristic of polymeric materials for tissue engineering is their permittivity. Relative permittivity is a coefficient of proportionality between electric charge and electric field and reflects how much the related charges can shift or be polarized under electric field action. It is known [24], that the relative permittivity of human derm, the main skin component, is from 10^6 to 10^2 in a frequency range from 1 kHz to 1 MHz, while for epidermis, the upper skin layer, the permittivity is constant and equal to 10^4.

The results of the measurements of dielectric characteristics of composite films based on chitosan and SWCNT are presented in Fig. 4.

Dependencies of $\operatorname{tg}\delta(f)$ (Fig. 4) show, that pure chitosan films have the lowest losses of 0.03 at 1 kHz; with SWCNT concentration increase the dielectric losses increase to 0.68. Dielectric losses of film with 1% SWCNT sharply increase due to high conductivity. Maximum in Fig. 4 (curve *5*) is most likely related to the Maxwell–Wagner–Sillars (MWS) polarization [25]. Interaction of SWCNT and chitosan can be explained with the model, presenting the microcondensers system, where chitosan is dielectric, while SWCNT is condenser armature. This model is described in the work [26], where the results of experiments and modelling using MWS are presented.

As per Fig. 5, with SWCNT concentration increase within 0–0.5 wt.% the permittivity at 1 MHz increases from 4.4 to 5.7. Increase of tubes content to 1 wt.% results in significant increase of permittivity at low frequencies, while its value reduces with the field frequency increase.

As known, under oscillating electric field exposure the polymer macromolecules are polarized [27]. Introduction of anisometric carbon particles with paramagnetic properties to polymeric dielectric matrix results in appearance of the second radiation, that forms in interaction with their external field [25]. Presence of SWCNT, that are paramagnetic like all coal-fiber materials, in the chitosan matrix contributes to polarization increase due to the secondary radiation. This lowers dielectric properties of the composite material, contributes to increase of its electrical conductivity, that is confirmed with data, presented in Fig. 3.

Dissipative processes of absorption and dispersion of both primary and secondary electromagnetic radiation result in significant increase of permittivity of the film, containing 1.0 wt.%, at low frequencies compared to pure chitosan film. With increase of radiation frequency in a range from 10^3 to 10^6 Hz the permittivity of the composite film decreases. Results on measurement of permittivity are

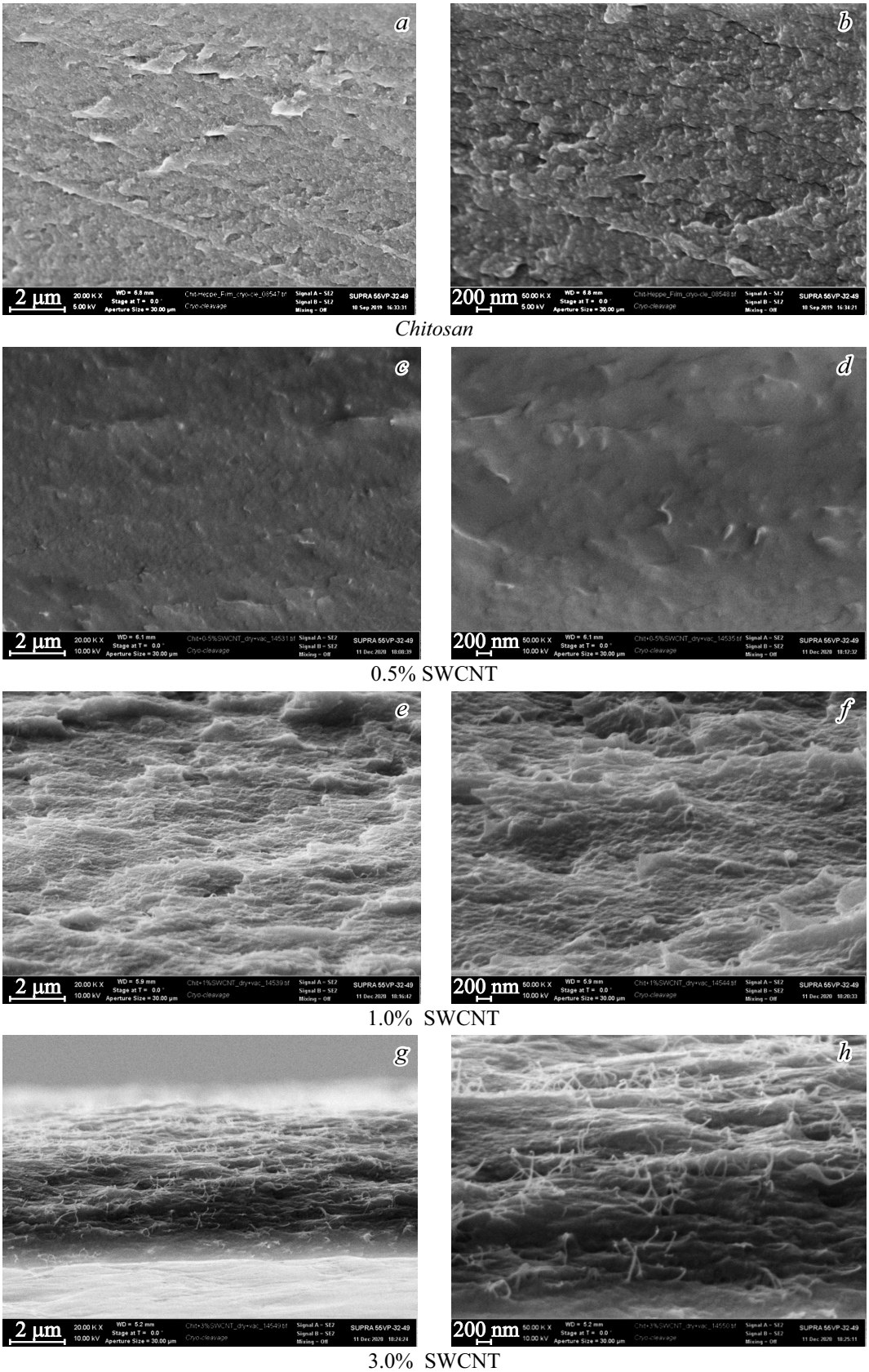

Figure 2. Microphotographs of shears in liquid nitrogen of chitosan film (a, b) and composite films, containing SWCNT $(c-h)$.

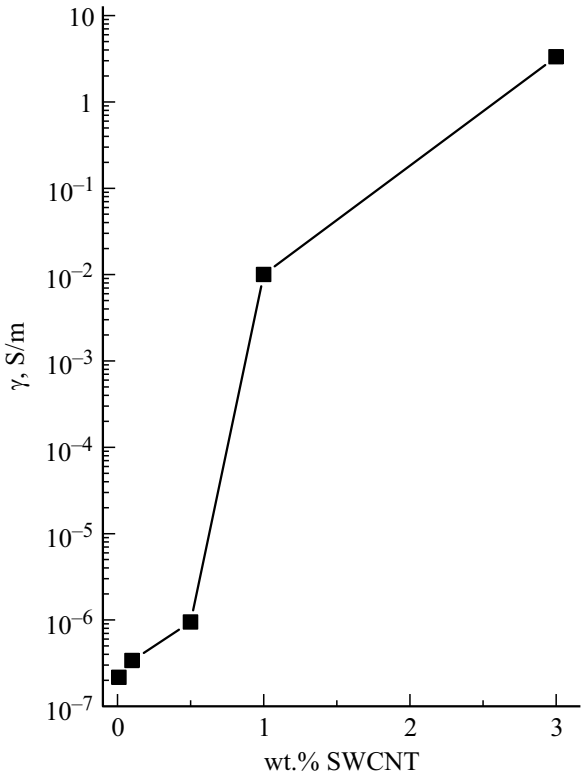

Figure 3. Change of volume electrical conductivity of composite films based on chitosan on SWCNT content.

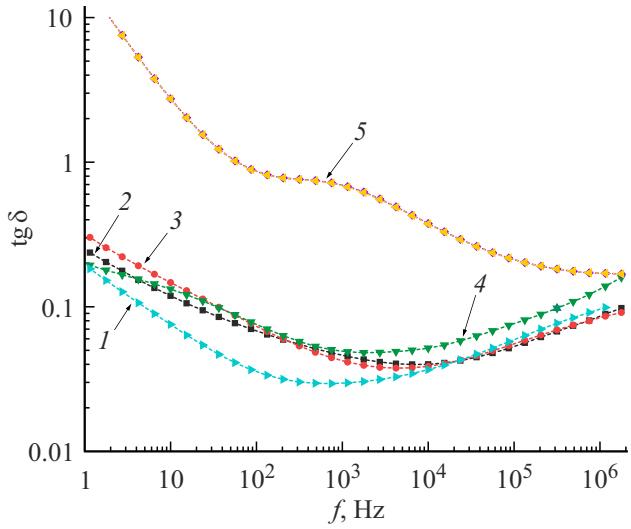

Figure 4. Dependence of a tangent of angle of dielectrical losses of chitosan films, containing 0 (*1*), 0.01 (*2*), 0.1 (*3*), 0.5 (*4*), 1.0 wt.% (*5*) SWCNT, on electric field frequency.

agreed with data, presented in the work [28] for pure chitosan. Figure 5 shows that ε' of chitosan is from 4.5 to 7 in a frequency range from 1 Hz to 1 MHz. Introduction of SWCNT into chitosan matrix allows to reach higher values of permittivity from 5.5 to 26 at 1 kHz, that can significantly increase compatibility of the implantable film and skin.

Effective use of biomedical materials assumes the possibility of their sterilization. One of the most common standardized sterilization methods is autoclaving under heating to 121°C, at pressure of 1 atm, for 30 min. Therefore, this work includes study of the thermal properties of the composite films of chitosan, containing SWCNT.

On the differential curve of chitosan DTG (Fig. 6) two areas of mass loss can be singled out: 50–150°C — area responsible for loss of moisture and residual solvent, and 260–360°C — area responsible for chitosan destruction process.

Data from TGA experiment with calculated values of thermal resistance indices (τ_5, τ_{10}) are presented in Table 1.

Thermal resistance was evaluated as per values of indices of thermal resistance τ_5 and τ_{10} to temperatures, at which 5 and 10% mass loss happens. It should be noted, that all films are characterized with high thermal stability. Temperature of their decay start is 277–289°C. These temperatures are significantly higher than temperatures of

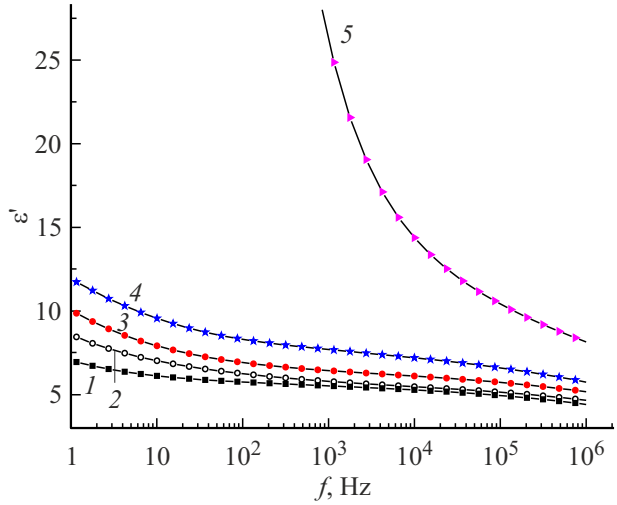

Figure 5. Dependence of relative permittivity of chitosan films, containing 0 (*1*), 0.01 (*2*), 0.1 (*3*), 0.5 (*4*), 1.0 (*5*) wt.% SWCNT, on electric field frequency.

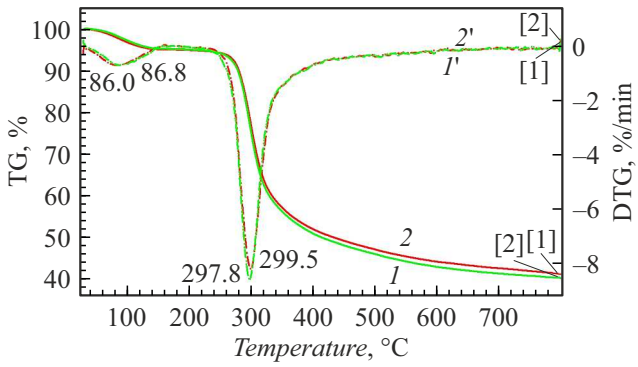

Figure 6. Thermogravimetric and differential thermogravimetric curves of the film of chitosan (*1*, *1'*) and composite film, containing 1 wt.% SWCNT (*2*, *2'*).

Table 1. Thermal properties of composite films based on chitosan and SWCNT

№	Content of SWCNT, %	Remaining mass (at 800°C), %	τ_5, °C	τ_{10}, °C
1	0	40.13	277	287
2	0.1	40.47	278	286
3	0.5	41.35	278	287
4	1	41.25	280	289
5	3	41.47	279	288

the standard sterilization modes. Introduction of heat-resistant carbon filler does not impact the composite thermal characteristics. Therefore, the chitosan films, containing SWCNT, can be heat treated, including sterilization at temperatures up to 250°C.

Table 2 contains the results of the mechanical tests of the composite films of chitosan–SWCNT.

Introduction of SWCNT results in strength increase and, what especially important, brittleness reduction, that is indicated with increase of relative deformation at rupture of the composite films. Content of more than 1 wt.% SWCNT reduces the composite film strength, that is related to presence of particles, oriented both in the film plane and perpendicular to it, that is shown in Fig. 2, h.

It should be noted, that implants of various purpose, as well as tissue-engineered structures, implanted into living organism, are subject to dynamic loads, caused by pulsating flow of liquid media (blood and lymph), as well as at reversal loads, tension and compression of muscular tissue. Therefore, it is important to know not just mechanical characteristics in tension or compression in stationary mode, but also the mechanical characteristics of samples in dynamic loading mode.

Figure 7 shows data of DMA samples, observed at loading frequency $\nu = 1$ Hz, deformation amplitude of 0.1%. It is seen, that with SWCNT concentration increase the elastic modulus $E'(T)$ increases from 3 to 4 GPa at temperature of 22°C. Temperature dependencies of elastic modulus demonstrate sharp drop in the region of defrosting temperatures of chitosan segmental mobility (246°C). Elastic modulus increase to 100°C and breaks in this temperature region are related to hygroscopic water loss [29].

Curves tg δ (Fig. 7) are characterized with wide maxima at temperature of 250–300°C, that define the glass transition temperature of the composites T_g. With SWCNT concentration increase the glass transition temperatures insignificantly shift towards high-temperature region. However, the thermal breakdown of chitosan is observed in the same temperature range. As per literature data [29], T_g of chitosan is close to its thermal destruction temperature. Temperature of the start of thermal destruction is (as shown above) in a range of 270–280°C for all examined samples.

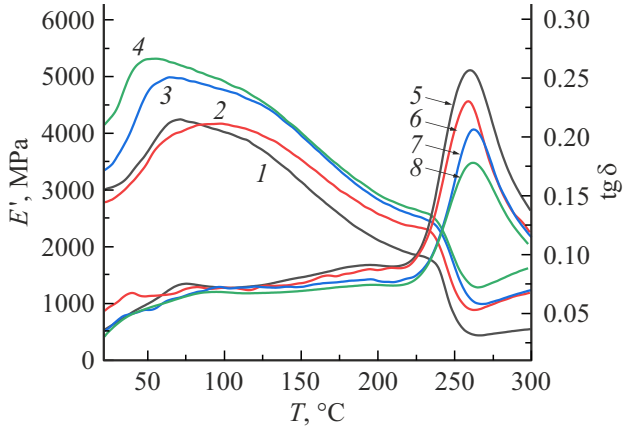

Figure 7. Dependence of elastic modulus E' of chitosan films, containing 0 (*1*), 0.5 (*2*), 1 (*3*), 3 (*4*) wt.% SWCNT, and the corresponding tangent of angle of mechanical losses (*5*–*8*) on temperature.

Conclusion

SWCNT presence in the chitosan film contributes to chitosan structure ordering and formation of more dense packing of macromolecules, resulting in increase of mechanical characteristics of the composite films. It is shown, that with SWCNT content increase to 1 wt.% the specific volume electrical conductivity increases to 10^{-2} S/m. Increase of permittivity in the range of low frequencies of 1 Hz is primarily caused by MWS polarization. Due to introduction of SWCNT the electrical and mechanical properties of the composites can be varied. Study of strength, deformation and thermal characteristics of the composite films allowedto make a conclusion on possibility of their use in cell technologies, tissue engineering, as well as for development of medical devices for diagnostics and treatment of diseases of various origin.

Table 2. Mechanical characteristics of the composite films of chitosan–SWCNT

Film	Tensile strength, MPa	Elastic modulus, GPa	Deformation, %
Chitosan	124.17 ± 5.42	2.62 ± 0.57	38.68 ± 4.84
Chitosan+0.1%SWCNT	157.74 ± 15.40	3.23 ± 0.24	46.09 ± 8.13
Chitosan+0.5%SWCNT	179.40 ± 5.40	3.22 ± 0.37	57.64 ± 3.57
Chitosan+1%SWCNT	161.04 ± 7.98	3.35 ± 0.27	47.14 ± 2.33
Chitosan+3%SWCNT	158.91 ± 17.23	3.57 ± 0.26	41.16 ± 5.82

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Conflict of interest

The authors declare that they have no conflict of interest.

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