The study of the properties of chitosan-based composite films filled with single-walled carbon nanotubes (SWCNTs) and treated with cold atmospheric plasma

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Abstract. In the work composite films based on chitosan and single-walled carbon nanotubes (0.01%, 0.1%, 0.5% and 1%) were obtained. The electrical and mechanical properties of the composite films were measured. It was shown that a film with 1% SWCNT has sufficient electrical conductivity and optimal mechanical properties for the bioactivity of a culture of human dermal fibroblasts. It has been established that treating a film in a corona discharge changes the structure of the film and increases the adhesion and proliferation of human dermal fibroblast cells.

1. Introduction
The study of cell behaviors on biomaterials surface is of paramount importance because it can reveal many physiological and pathological events and eventually guide the design of biomaterials with better performance in tissue regeneration. Presently, the functionalization of biomaterials is one of the emerging processes. Among all composites the nanocomposites are on the forefront of research and technology development especially for therapeutic purposes. Technically, nanocomposites are comprised of biopolymers instead of synthetic and/or petroleum-derived polymers. More often, they are classified into a specific classis of materials referred as “bionanocomposites” are the nanomaterials which implicate a biopolymer of natural origin in combination with an inorganic moiety, and display different in properties like biocompatibility and biodegradability [1].

Chitosan (CS), a promising biomaterial that by itself presents outstanding properties, revealed to improve the nanotechnology field when reinforced with various other nano-sized fillers. Depending on the application, the characteristics of each CS nanocomposites can be controlled, designed and modulated regarding the target tissue. In the presence of fillers like single-walled carbon nanotubes (SWCNTs), enhanced mechanical, thermal and conductive properties are achieved.

In addition, it is known that non-thermal effects of gas discharges have proven to be very useful in the treatment of different biomaterials and in tissue engineering. Cold atmospheric plasma (CAP) is very far from thermodynamic equilibrium. Particularly, the electron temperature is about 10 000 K and significantly exceeds the gas temperature, which is close to room temperature. Besides that, cold plasma is a source of energetic and chemically active species (e.g., electrons, ions, excited atoms, and radicals). Owing to these features, CAP is used in the treatment of polymers and other temperature-sensitive materials.

2. Materials and methods

2.1. Preparation of composite films
Composite films were prepared from 4% chitosan (Biolog Heppe GmbH, Germany, $M_w=(1.64-2.1)\times10^5$, DD=92%) solutions containing SWCNTs (Carbon Chg, Russia) in 2% acetic acid solution. The content of SWCNTs with respect to chitosan was 0.01; 0.1; 0.5; 1 wt. %.
Before the preparing solutions, a suspension containing SWCNTs was dispersed to uniformly distribute particles throughout the volume. This was done using (1) an ultrasonic bath Sapphire for 30 min at a frequency of 35 kHz and power of 190 V and (2) an ultrasonic generator IL10-0.63 for 7 min at a frequency of 25 kHz and power of 630 V.

Chitosan was introduced into the obtained aqueous dispersion in the amount that provided the polymer concentration in the solution equal to 4.0 wt.%. This is the optimal concentration of polymer for coagulation spinning of chitosan fibers [2]. The mixture of chitosan and SWCNTs was stirred in water for 30 min in order to achieve swelling and partial dissolution of chitosan. Then acetic acid was introduced into the system; its concentration was brought to 2%. The solution was stirred for 90 min, then filtered and deaerated in a vacuum chamber for 24 hrs at a pressure of 10 kPa.

The films were cast by extruding the solution through a slit die onto a glass substrate; the casting was followed by drying at room temperature for 24 hrs. Film thickness was 40 ± 10 µm. The films prepared by dry-casting method were exposed to 10% aqueous solution containing NaOH and C2H5OH (1:1) for 10 min, then washed with distilled water and dried in air [3, 4].

2.2 Cold atmospheric plasma treatment
A custom-designed portable CAP generator (figure 1) was used for polymer composite samples’ processing. In contrast to a number of known types of plasma sources, this generator produces a stable air plasma using a self-sustained corona discharge of positive polarity (input voltage is 5.3 V while the output is about 15-16 kV, discharge current amplitudes are ranging from 10 up to 80 mA with a repetition rate of several tens of kilohertz, the average discharge current is about 100 – 150 μA). In the experiment, the samples were processed by the CAP at a distance of 1 cm for 10 min.

![Figure 1. A custom-designed portable CAP generator](image)

2.3 Electrical conductivity measurement
The electrical conductivity of films was studied in isothermal conditions at 25°C. The currents were measured using a picoammeter A2-1 with a two-electrode system. The sensitivity of the picoammeter A2-1 is 10^-15 A. The measurement error of current is 1%. The films were coated with platinum. The diameter of the electrodes was 16 mm. The charging currents were measured on constant voltage 10 V, electrical field strength was ~2.5*10^5 V/m. The time of current measurement was 1 minutes. The film with 1% SWCNT content was measured using four electrode systems.

2.4 Mechanical testing of SWCNTs -chitosan composite film
The measurements of the mechanical properties of the films were carried out with Instron 5943 (USA) at room temperature in the mode of stretching the sample in the form of strips 2 mm wide and 30 mm long. Tensile tests were carried out at a speed of 5 mm/min at room temperature. Prior to the mechanical testing films were stored at a relative humidity of 66% for 24 hours.
2.5 Contact angle measurement
Contact angles of chitosan and composite chitosan-based films were measured by the “sessile drop” method with the drop shape analyzer DSA30, Kruss (Germany). Distilled water was used as a test liquid. 5 measurements were taken at each point.

3. Results and discussions

3.1 Mechanical properties of SWCNTs -chitosan composite films
The mechanical properties of the composite films are shown in the table 1. The values of the mechanical properties of chitosan-based composite films are obtained depending on the concentration of SWCNTs in the composition.

| Table 1. Mechanical properties of SWCNTs -chitosan composite films |
|-------------------------------------------------------------|
|               | Tensile strenght, MPa | Elastic modulus, GPa | Elongation at break, % |
| chitosan        | 128,5±12               | 4,7±0,12               | 20,4±6,9                |
| chitosan+0,01% SWCNTs | 121,58±0,9             | 4,47±4,5               | 17,38±3,4               |
| chitosan+0,1% SWCNTs | 109,1±12               | 3,9±0,37               | 20,9±7,3                |
| chitosan+0,5% SWCNTs | 141,1±0,3              | 4,8±1,1                | 30,5±5,8                |
| chitosan+1% SWCNTs | 112,3±15               | 4,4±0,16               | 19,4±6,5                |

The addition of 0.1% SWCNTs to chitosan films reduces the mechanical characteristics of the composition as a result of the formation of local defects. Molecular chain reorientation occurs relative to SWCNTs added into chitosan solution, but the amount of SWCNTs is insufficient to establish a regular cluster structure. The establishment of a regular cluster structure occurs when ≤ 0.5% SWCNTs are added to the chitosan film, which leads to increase in strength and elongation at break of the composite films, meanwhile the modulus remains unchanged. The addition of 1% SWCNTs to chitosan films again reduces the mechanical characteristics of the composition, which is probably due to the high content of SWCNTs in the composition and coagulation of particles.

3.2 Contact angles of SWCNTs -chitosan composite films
The contact angles chitosan films filled with SWCNTs at a concentration of 1 wt% with respect to chitosan were measured without treatment and after treatment CAP (table 2).

| Table 2. Contact angles of SWCNTs -chitosan composite films |
|-------------------------------------------------------------|
| Before CAP treatment, grad | After CAP treatment, grad |
| chitosan        | 69±0,5               | –                      |
| chitosan+0,01% SWCNTs | 66±0,5               | –                      |
| chitosan+0,1% SWCNTs | 69±1,0               | –                      |
| chitosan+0,5% SWCNTs | 70±1,0               | –                      |
| chitosan+1% SWCNTs | 68±0,5               | 61±0,5                |

Processing with CAP leads to a decrease in the hydrophobicity of the films: the contact angle of the composite films decreases from 68 ° (before processing) to 61 ° (after processing).
3.3 The dependence of the electrical conductivity of composition films

Figure 2 shows the change in the electrical conductivity of chitosan films depending on the content of single-walled carbon nanotubes in them. The value of the electrical conductivity of unfilled chitosan is $10^{-13}$ S/cm [5].

![Figure 2. Volume resistivity of chitosan-based composite films filled with SWCNTs.](image)

From the dependence, we can distinguish a percolation cluster, which determines a sharp decrease in electrical resistivity $10^2$ Ohm*m. The threshold for the flow of electric current is 0.6-1% SWCNTs.

3.4 Fibroblast attachment, viability, proliferation and morphology

After cell attachment, human dermal fibroblasts seeded on film matrices and cultural polystyrene surface (control) were incubated in culture medium at 37°C in a humidified 5% CO2 incubator for 4 days. Studies of the viability and proliferation of the cells in the samples were performed using the MTT test.

![Figure 3. Human dermal fibroblast viability and proliferation on surfaces of film matrices and cultural polystyrene surface (control) as determined by MTT assay](image)

The proliferation rate of cells (for 4 days) on treated film matrices was found to be greater than on untreated film matrices (Fig. 3).
It has been shown that the filler addition and CAP treatment increase the conductivity of the composite material. By changing the conductivity, it is possible to obtain matrices with different bioactive properties.

4. Conclusion
In the work, new composite films based on chitosan and single-walled carbon nanotubes were studied. It was shown that a film with 1% SWCNT has sufficient electrical conductivity and processing with CAP leads to a decrease in the hydrophobicity of the films, which contributes to an increase in their biological activity.

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