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Preparation and cytotoxicity of chitosan-based hydrogels modified with silver nanoparticles

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ABSTRACT

Chitosan based hydrogels are commonly applied in various fields of medicine and pharmacy. Modification of hydrogel polymers using nanosilver particles may result in formation of materials with enhanced antibacterial properties. In this article synthesis of hydrogel materials based on chitosan and modified with silver nanoparticles is presented. First, preparation and characterization of silver nanoparticles using UV–vis spectroscopy has been shown. Hydrogels modified with nanosilver particles were subjected to the measurements of swelling ability and *in vitro* tests in distilled water and Simulated Body Fluid (SBF), respectively. Additionally, evaluation of antibacterial properties against *Staphylococcus aureus* and *Enterococcus faecalis* as well as results of cytotoxicity of hydrogel materials modified with silver nanoparticles conducted by means of XTT and MTT assays using dermis cells BJ (CRL-2522TM) have been presented.

1. Introduction

Nowadays, nanotechnology becomes more and more popular [1]. Metallic nanoparticles such as silver- or gold nanoparticles are applied in many fields including medicine or pharmacy. These nanosized particles are well-known substances used in biomedical applications. As it has been previously reported nanosilver is characterized by antibacterial properties. It contributes to the destruction of sulfur and phosphorus compounds in proteins or DNA contained in cell structure leading to cell death [2,3]. Due to the negative impact on the microorganisms, described nanoparticles are also applied in preparation of modern wound dressings. Recently, Anjum et al. [4] reported novel wound care systems obtained on the basis of many compounds including nanosilver. Furthermore, particles introduced into the hydrogel matrix are also applied as biosensors [5]. It is worth noting that due to their antimicrobial activity, silver nanoparticles are also used in food industry as components of modern food packaging. According to Castro-Mayorga, nanocomposites on the basis of stabilized silver nanoparticles can be an alternative to currently existing food packaging [6]. That protects food from undesirable microorganisms prolonging its shelf life. Furthermore, a certain amount of nanosilver is used during the synthesis of plant packaging based on polyethylene [7].

In the present paper chitosan has been considered as a matrix of hydrogel materials. This naturally occurring biopolymer is widely used in many realms including medicine, pharmacy, food industry or environmental protection. Due to its therapeutic properties that polysaccharide is used as a component of modern dressings accelerating healing process [8–11]. Abdel-Rahman et al. describe a synthesis of wound dressings on the basis of chitosan in combination with sodium hyaluronate and nonwoven cotton fabrics. These materials were characterized by antimicrobial activity in contact with microorganisms such as *Escherichia coli* or *Staphylococcus aureus* [12]. Moreover, Anjum et al. reported that application of hydrogel dressings based on chitosan can prevent the formation of scars [13].

Discussed materials belong to the group of polymers known as hydrogels. These materials due to their unusual properties are widely applied in a wide variety of areas. Their characteristics - biodegradability, biocompatibility or bioactivity - are essential from a medical point of view. It is worth noting that different substances can be introduced into the matrix of the mentioned polymers leading to an enrichment of the material with new properties [14–17].

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The present paper constitutes the second part of our research dedicated to chitosan-based hydrogels containing nanosilver. The work is focused on the determination of sorption ability of these materials in distilled water and in SBF as well as on the impact of the hydrogels on these liquids. Furthermore, research on the antibacterial activity of the hydrogels in relation to Staphylococcus aureus and Enterococcus faecalis has been carried out, and the cytotoxicity of the materials towards cells of the dermis has been investigated. In previous work [28], the swelling properties and behavior of similar materials in Ringer's liquid and in artificial saliva solution were determined as well as their cytotoxicity towards epidermal cells and their antibacterial activity in relation to Escherichia coli. Spectroscopic analysis of the hydrogels as well as their surface morphology were also reported previously [28].

2. Materials

All chemicals applied in the research were of analytical reagent grade. The silver nitrate (99.9% $AgNO_3$, pure p.a., catalogue number: 814322777), sodium borohydride (98% $NaBH_4$, pure p.a., catalogue number: 793400110) and gelatin (pure p.a., catalogue number: 901946119) were bought in Avantor Performance Materials Poland (formerly POCH S.A.). Materials such as 2-hydroxy-2-methylpropiophenon (catalogue number: 7473-98-5, photoinitiator), poly(ethylene glycol) diacrylate Mn = 700 (catalogue number: 26570-48-9, crosslinking agent) and chitosan low molecular weight (catalogue number: 9012-76-4) were supplied by Sigma Aldrich.

3. Experimental part

3.1. Preparation of silver nanoparticles

Silver nanoparticles were prepared by the chemical reduction process [18–22]. Silver nitrate was used as a source of silver ions, while sodium borohydride was a reducing agent. The reduction was carried out in the presence of stabilizing agent – poly(vinylpyrrolidone) (PVP, M_w = 8 000). Concentration of silver nanoparticles amounted to 250 ppm. Detailed description concerning preparation of silver nanoparticles solution is presented in the Supplementary Material.

3.2. Preparation of hydrogels modified with silver nanoparticles

Synthesis of hydrogel materials requires a few steps. First of them is dissolution of the appropriate amount of chitosan and gelatin in 0.05% acetic acid solution. Then, silver nanoparticles solution (at a concentration of 250 ppm) and reagents including crosslinking agent and photoinitiator (amounts indicated in Table 1) are added and the whole mixture is treated with UV radiation for 2–3 min (lamp: EMITA VP-60 with the following parameters: power - 120 W, applied wavelength: λ = 320 nm). More detailed description of the conducted synthesis is presented in the Supplementary Material.

Subsequently, hydrogels obtained were subjected to the numerous studies in order to determine their physicochemical properties.

3.3. Characterization of silver nanoparticles

3.3.1. UV–vis spectrophotometry

UV–vis spectroscopy measurements (330–700 nm) were performed using Specord 205 with a 1-cm optical length cuvette and a spectral resolution of 1 nm. The study was conducted at room temperature.

3.4. Studies on prepared hydrogels modified with silver nanoparticles

3.4.1. Swelling studies

A well-known feature of hydrogels is their ability to absorb fluids in a reversible manner. Therefore, materials based on chitosan and containing silver nanoparticles were subjected to the swelling studies. In order to check their swelling ability samples of synthesized materials were immersed in fluids such as distilled water and simulated body fluid (SBF, pH = 7.40) for scheduled period of time. Samples, after immersion in solutions for 1, 24 or 72 h, were separated from the solution in the swollen state, and weighed. Sorption capacity was determined by calculating the swelling ratio *Q [g/g]* defined based on the following equation:

$$Q = \frac{w - w_0}{w_0}$$

where:

w – weight of swollen sample [g], w_0–hydrogel weight before immersing in solution [g].

More detailed description of the procedure of swelling studies is presented in the Supplementary Material.

3.4.2. Incubation studies

Materials assigned for biomedical purposes should not affect harmfully the human body or disrupt its homeostasis. Thus hydrogels have been immersed in distilled water and in SBF for a period of about 21 days. The change of pH of the fluids were measured every two days.

3.5. Determining of antibacterial activity

Strains from the collection of reference strains of *Staphylococcus aureus ATCC 25923* and *Enterococcus faecalis ATCC 29212* were used for research. Suspension of microorganisms of 0.3 McFarland density was inoculated on a plate with the substrate – Mueller Hinton agar. Then, samples of hydrogel dressings with a diameter of 12 mm were cut out using cork borer and placed in the previously prepared Petri dishes. Three samples for each strain were used. Samples of hydrogels were incubated on Petri dishes at 37 °C respectively for 1, 2 and 6 h. After 1, 2 and 6 h samples were removed and Petri dishes were further incubated for 24 h.

After incubation time, presence of bacteria under the hydrogel sample was checked. The number of colonies grown was counted and compared with the control test. The number of colonies in the control group was assigned as 100%. The effect of the antibacterial activity on the tested substance was expressed in percent:

$$(\%) = (x/y)100,$$

where:

x - number of colonies in tested sample,

y - number of colonies in control sample.

Results of conducted studies were summarized on the basis of (%) obtained as below.

> 90% growth inhibition = strong antibacterial effect

50 to 90% of inhibition = poor antibacterial effect

< 50% growth inhibition = no antibacterial effect

The sample with colonies grown in the control test was prepared using 100 μl NaCl instead of hydrogel tested. The rest of the preparation procedure was the same as in the case of the tested hydrogels. Research were carried out 3 times for each tested model. The study was conducted in accordance with the procedures recommended by PN-EN 1040 and BS EN 1276 standards.