Advanced Biomaterials with Semiconductive Properties Based on Fungal Chitosan

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Abstract

Tissue engineering is a branch of science that focuses on methods and techniques for the creation of new tissues and organs for the therapeutic reconstruction of the damaged organ by providing support structures, cells, molecular and mechanical signals for regeneration to the desired region. Conventional implants made of inert materials can eliminate only physical and mechanical defects of damaged tissues. The goal of tissue engineering is to restore biological functions, that is regeneration of tissues, and not only to replace it with a substitute made of synthetic material. The most important challenges of tissue engineering include the development of new biomaterials that will be used as three-dimensional scaffolds for cell cultures. Such scaffolding must be characterized by biocompatibility and biodegradability.

The aim of the research was to obtain biomaterials based on acylated chitosan. The result of the work was to obtain three-dimensional scaffolding with bioactive properties based on raw materials of natural origin. The biomaterials were modified with ferrimagnetic nanoparticles which are capable of electromagnetic stimulation of proliferation.

Keywords: *waste biomass, chitosan, semi-conductive materials, Green Chemistry, scaffolds*

Introduction

Regenerative medicine is an alternative to traditional transplantation. It enables obtainment of tissues under in vitro conditions using primary cells derived from the patients. Cells are cultured on three-dimensional scaffolds which mimic their natural environment. The substrates can be prepared from synthetic polymers and biopolymers. Natural polymers are known of their excellent biocompability and biodegradability. However, their mechanical durability is quite low. Thus biomaterials prepared from them are modified with various substances both organic and inorganic. An interesting approach constitues the application of nanoparticles to enhance raw polymer properties [PIĄTKOWSKI, Marek et al., 2019].

Nanotechnology is currently the most dynamically developing field of science. Rapid development is caused by great interest, financing of many studies and the possibility of using nanomaterials in almost all fields of science and everyday life. The goal of nanotechnology is to obtain new nanomaterials with the desired properties. Iron oxide nanoparticles, thanks to their size similar to the size of cells and due to their superparamagnetic properties, have been used in diagnostics (enhancing contrast in magnetic resonance imaging), therapies (hyperthermia, drug delivery) and industry (bioseparation) [FATIMA, Hira et al. 2018, LAURENT, Sophie et al. 2008, SIMONSEN, Galina et al. 2018]. There are two possibilities for obtaining nanoparticles. The first of these is the "top-down" method consisting in reducing the particle size of classical matter by, for example, grinding in a mill. This process is long-term, time-consuming and energy-intensive. The second and more common approach is the "bottom-up" method, which consists in the production of liquid-phase nanoparticles. The most commonly used syntheses of this type include: coprecipitation, sol-gel, colloidal and solvothermal methods. Coprecipitation is a simple process. It consists of adding a basic alkaline reagent to aqueous salt solutions. The pH then rises and new seeds are formed, then they grow and age until the saturation level is reached. In the sol-gel method, the sol should first be obtained, a suspension in which the solid particles are not sedimented. Precursors including inorganic metal salts, metal alkoxides, acetates together with the solvent form a sol that then turns into a gel. The solvothermal methods consist in carrying out chemical reactions at a temperature exceeding the boiling point of the solvent and at elevated pressure. They are most often used to change the amorphous material into a crystalline one [CHEN, Zhou et al. 2018, R.V. MEHTA, 2017].

In order to give certain properties, reduce the tendency to agglomerate, protect against environmental factors and increase biocompatibility, magnetic nanoparticles are functionalised by surface coating using suitable polymeric stabilizers or surfactants, e.g. polyvinyl alcohol (PVA), polyethylene glycol (PEG), or by depositing several atomic metal layers (e.g. gold) or non-metals (e.g. graphite); the production of polymeric coatings that prevent the growth of clusters after nucleation and keep the domains of particles away from the attraction forces; by creating lipid coatings around the magnetic core [REDDY, Harivardhan et al. 2012].

Chitosan is a natural polymer known of its cytocompability and biodegradability. The biopolymer is built from two type of mers – aminoglucose and N-acetylaminoglucose. It may undergo numerous chemical and physical modifications due to presence of free hydroxy and amino functional groups. Untill now, the scientiscts were focused on the application of chitosan obtained from shrips, crabs and lobsters. However, the potential of fungal chitosan is still not fully discovered. Fibroblasts are the most common cells of the connectvie tissue. They are a building part of skin (dermis). They are responsible for extracellular ma-

Tab. 1. Chitosan scaffolds synthesis parameters
Tab. 1. Parametry syntezy rusztowań chitozanowych

Sample	Chitosan, g	Aspartic acid, g		Propylene glycol, ml	Fe_3O_4 NPs, %	Time, min	Temperature, °C
1	0.50	0.74		7		45	120
2					0.5		125
3							130
4							135

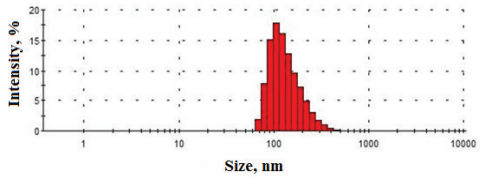

Fig. 1. The particle size distribution of obtained sample using ultrasounds without the addition of polimer
Rys. 1. Rozkład wielkości cząstek uzyskanej próbki za pomocą ultradźwięków bez dodatku polimeru

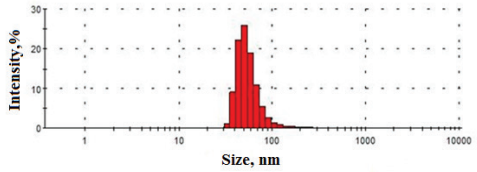

Fig. 2. The particle size distribution of obtained systems using ultrasounds stabilized by 0.1 mL PVA 14000
Rys. 2. Rozkład wielkości ziaren otrzymanych z wykorzystaniem ultradźwięki stabilizowane 0,1 ml PVA 14000

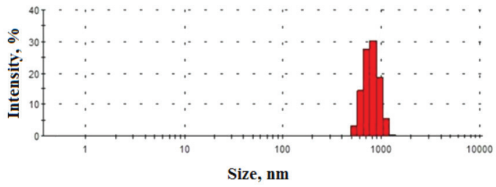

Fig. 3. The particle size distribution of obtained systems using ultrasounds stabilized by 0.2 mL PVA 14000
Rys. 3. Rozkład wielkości cząstek otrzymanych z wykorzystaniem ultradźwięków stabilizowanych 0,2 ml PVA 14000

trix components secretion [BALAKRISHNAN, Biji et al. 2011, PIĄTKOWSKI, Marek et al., 2019, SHAHID, Mohammad et al.2013, SHAMSHINA, Julia et al. 2019, VLIERBERGHE, Van et al. 2011]. In this article, a novel strategy of bioactive scaffolds syntehsis strategy is presented using fungal chitosan as a raw material and ferrimagentic.

Materials and methods

Materials

Chitosan was purchased from Polaura, Poland. The iron (III) chloride, ammonium and iron (VI) sulfate, ammonia, sodium citrate, poly(vinyl alcohol), poly(ethylene glycol), poly(vinylpyrrolidone) were received from Avantor Performance Materials Poland. All compounds were characterized by analytical purity. L-Aspartic acid, fibroblast growth medium (FGM) and human dermal fibroblasts (HDF) were purchased from Sigma Aldrich, Poland. Ethanol was purchased from Avantor, Poland.

Methods

Biomaterials preparation pathway

The solutions were prepared by dissolving 0.584 g $FeCl_3$ and 0.423 g Mohr salt in 100 mL of distilled water. 10% stabilizer solutions were prepared by dissolving 5 g of polymer in 45 g distilled water. Three methods of energy supply were used in the experiment: a heating plate, a microwave reactor and an ultrasonic cleaner with a heating function. Various volumes of stabilizers were added to the precursor solutions. The whole was heated to 60°C, and then 15 mL of a 5% ammonia solution was added dropwise. The produced precipitate was rinsed twice with distilled water to get rid of the salts which had not reacted. Then, aspartic acid was dissolved in the distilled water containing Fe_3O_4 NPs. Next, chitosan and propylene glycol were added and reaction was carried out under microwave radiation using Prolabo Synthwave reactor. Finally, the products were lyophilised.

DLS analysis

DLS analysis was performed using zeta sizer analyser.

FT-IR analysis

FT-IR analysis was performed using IR Thermo Nicolet Nexus X 470 spectrometer (diamond crystal ATR), USA. The range was between 400 and 4000 cm^{-1} with 32 scans and 4 cm^{-1} resolution.

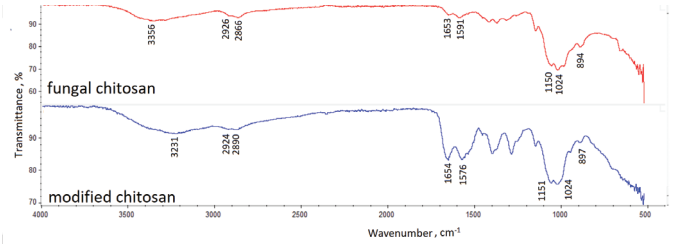

Fig. 4. FT-IR spectra of the prepared biomaterials
Rys. 4. Widma FT-IR przygotowanych biomateriałów

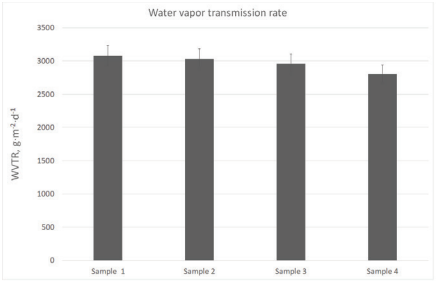

Fig. 5. Water vapor transmission rate of the prepared biomaterials
Rys. 5. Szybkość przenikania pary wodnej przygotowanych biomateriałów

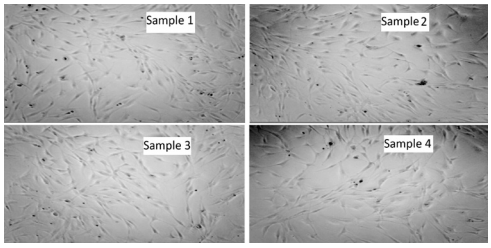

Fig. 6. Human dermal fibroblasts after cell culture in the presence of the prepared biomaterials
Rys. 6. Ludzkie fibroblasty skórne po hodowli komórkowej w obecności przygotowanych biomateriałów

Water vapor transmission rate (WVTR)

Water vapor transmission rate was performed by placing weighed samples on the vessels containing 10 mL of distilled water. The biomaterials were fixed with glue to cover the container. The vessels were left for 24h at 37°C. Next, the amount of the evaporated water was determined. To calculate WVTR the following equation was used:

$$WVTR=(W_t - W_0)/(tA)\ )(g \bullet m^{-2} \bullet d^{-1})$$

where: W_t – the weight after time t, W_0 – the initial weight, t – the measuring time, A – the area of the opening of polystyrene well

Cytotoxicity study

To verify cytotoxicity of the prepared biomaterials cell culture of the primary cells – human dermal fibroblasts was carried out. The culture was performed for 48h at 37°C and 95% CO_2 concentration. As a culture medium fibroblast growth medium was used. The cells were investigated under inverted microscope.

Results and discussion

DLS analysis

Figures 1–3 presents results of particles size determination by DLS method. For particles obtained in synthesis 1 had size of 100 nm, synthesis 2–50 nm, while in synthesis 3–900 nm.

Differences in the size of the obtained particles indicate the importance of choosing the right stabilizer concentration during the synthesis. In synthesis 2, the addition of 0.1 mL of PVA solution favored the formation of nanometric size particles. The increase in the stabilizer concentration decreased the scatter of results, increased the homogeneity of the obtained samples. This was due to the stabilizer properties, which adsorbed on the particles prevented their agglomeration.

FT-IR analysis

Figure 4 shows the chemical structure of the raw and modified chitosan. Pure polymer prepared from fungi shows all bands typical for chitosan coming from free hydroxyl and amino groups, alkyl groups, glucopyranose rings and glycosidic bonds. FT-IR spectrum of the modified chitosan proves acylation reaction occurrence since it can be noticed that new amide bonds were formed between polymer amino groups and carboxylic groups of aminoacid. At the same time, it can be noticed that the rest of the bands are similar which means that no degradation of chitosan took place. Thanks to the presence of the hydrophilic groups including amino, carboxyl and hydroxyl the ob-

tained material had a hydrogel nature and was capable of water sorption.

Water vapor transmission rate

Biomaterials dedicated to applications in skin tissue regeneration must meet various conditions, such as good water vapor permeability. Appropriate humidity and gas exchange are crucial for damage skin recovery. Results shown in Figure 4 indicate, that all prepared samples are permeable for water vapor in the similar manner. Such permeability can be caused by the very porous structure of the biomaterials which was achieved by the lyophilization of the swollen samples. After freezing, water molecules increased their volume creating spread pores in the polymeric matrix as a result of sublimation.

Cytotoxicity study

The proposed biomaterials are dedicated to tissue engineering applications and should provide appropriate environment for skin cells adhesion and multiplication. The raw chitosan is known of its biocompability. However, its chemical modification can deteriorate this feature. Also, the addition of nanoparticles may sometime induce undesired cellular responses, including apoptosis. Figure 6 presents results of cytotoxicity study carried out on human dermal fibroblasts. It can be observed that cells are of standard morphology and have spindle-like shape. Additionally, their nuclei are flat and oval and no grains in cytoplasm can be noticed. Therefore, it can be stated that the prepared biomaterials are non-cytotoxic. Additionally, comparing to the cells cultured without biomaterials it can be noticed that the proliferation activity is higher.

Conclusion

The aim of this research was to obtain novel type of material using chitosan prepared from fungi as a main component. Also, Fe_3O_4 nanoparticles were prepared under synthesis conditions. The polymer was successfully modified by acylation using L-aspartic acid and obtained ferrimagnetic NPs. Ready products were examined over their physicochemical properties. Finally, their biocompability and bioactivity were confirmed on human dermal fibroblasts. Overall, the proposed biomaterials have a great potential in tissue engineering, especially of skin tissue.

Acknowledgements

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Zaawansowane biomateriały o właściwościach półprzewodnikowych oparte chitozanie pochodzącym z grzybów

Inżynieria tkankowa jest dziedziną nauki, która koncentruje się na metodach i technikach tworzenia nowych tkanek i narządów do terapeutycznej rekonstrukcji uszkodzonego narządu poprzez dostarczanie struktur wspierających, komórek, sygnałów molekularnych i mechanicznych do regeneracji w pożądanym kierunku. Konwencjonalne implanty wykonane z materiałów obojętnych mogą wyeliminować fizyczne i mechaniczne wady uszkodzonych tkanek. Celem inżynierii tkankowej jest przywrócenie funkcji biologicznych, czyli regeneracja tkanek, a nie tylko zastąpienie jej substytutem wykonanym z materiału syntetycznego. Najważniejsze wyzwania inżynierii tkankowej obejmują rozwój nowych biomateriałów, które będą wykorzystywane jako trójwymiarowe rusztowania do hodowli komórkowych. Takie rusztowanie musi charakteryzować się biokompatybilnością i biodegradowalnością. Celem badań było uzyskanie biomateriałów na bazie acylowanego chitozanu. Rezultatem prac było uzyskanie trójwymiarowego rusztowania o właściwościach bioaktywnych na bazie surowców pochodzenia naturalnego. Biomateriały zmodyfikowano nanocząstkami ferrimagnetycznymi, które są zdolne do elektromagnetycznej stymulacji proliferacji.

Słowa kluczowe: *odpadowa biomasa, chitozan, materiały półprzewodnikowe, Zielona Chemia, rusztowania chitozanowe*