Bioactive Scaffolds for Skin Tissue Engineering Doped with Gold Nanoparticles Prepared from Waste Biomass

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Abstract

Skin is the first barrier against pathogens and harmful external factors. Each damage of this tissue may cause microbial infection and danger to internal organs. Burns which may be a result of the exposure to radiation, chemicals or high temperature leads to the significant disruption of skin functions. The most promising method for this tissue recovery is regenerative medicine which requires application of three-dimensional biocompatible scaffolds. The biomaterials enable skin cells proliferation and new tissue formation under in vitro conditions. They can be prepared from synthetic and natural polymers and their combination. The application of additional components such as nanoparticles may enhance their mechanical properties and have a positive impact on fibroblasts divisions and extra cellular formation. One of the most promising raw materials for scaffolds is chitosan -a chitin derivative. It may be obtained from waste biomass such as crabs, shrimps and lobsters exoskeletons. Chitosan is non-toxic, biodegradable and have antibacterial properties. The aim of the following study was to obtain novel chitosan derivatives doped with the gold nanoparticles using only natural components such as orange peels and fatty acid derivative. Proposed modification strategy resulted in the preparation of the novel, biodegradable and biocompatible material with interesting properties. The products were analysed by UV-Vis and FT-IR methods. The scaffolds were investigated over their susceptibility to enzymatic degradation. Finally, the biomaterials were verified over their cyto-compability with human dermal fibroblasts. The results showed that the proposed synthesis pathway resulted in the obtained of the chitosan biomaterials with high potential in medicine.

Keywords: *waste biomass, gold nanoparticles, chitosan, biomaterials, Green Chemistry*

Introduction

Annually, 20,000 patients await skin grafts as a result of burns. Currently, the only solution for these people are autogenous, allogenic and xenogeneic grafts. These types of treatments are associated with a limited amount of available tissue. Alternative to transplantation is regenerative medicine and tissue engineering. They enable the cultivation of skin cells on special substrates that can be transplanted to the patient [AMBEKAR, Rushikesh et al. 2019, BARGUES, L et al. 2011].

Cell cultures are carried out on three-dimensional scaffolds, which must be non-toxic and biocompatible, as well as allow the flow of nutrients. In order to ensure proper tissue regeneration, scaffolds should be characterized by controlled biodegradability under the influence of enzymes naturally occurring in the human body. They should also promote cells proliferation [AHMED, Shakeel et al. 2018, RADWAN-PRAGŁOWSKA, Julia et al. 2019].

Chitosan is a biodegradable polymer obtained as a result of deacetylation of chitin, which is a biopolymer present in exoskeletons of crustaceans constituting a large-scale food industry waste, especially shells of shrimps, crabs and lobsters [KNIDRI, Hakima et al.2018, VLIERBERGHE, Van et al. 2011].

Chitosan due to it favorable features such as biocompability and biodegradability is widely applied in medicine and pharmacy as a component of wound dressings or controlled drug delivery and release systems [MITTAL, Hemant et al.2018, SHARIATINIA, Zahra et al. 2019]. Gold nanopartilces which are particles below 100 nm are known of their positive impact of extracellular matrix components secretion, especially collagen [PIĄTKOWSKI, Marek et al. 2019, THAKOR, A. S. et al. 2011].

The aim of the research was to obtain new chitosan derivatives with controlled biodegradability, which can be used in regeneration of full skin thickness. The biomaterials were modified with gold nanoparticles which have positive impact on new extracellular matrix formation.

Materials and methods

Materials

Chloroauric acid, triethylamine, acetone, oleoyl chloride, fibroblast growth medium (FGM) and human dermal fibroblasts (HDF), simulated body fluid (SBF) and lysozyme were purchased from Sigma Aldrich, Poland. Acetone, ethanol and methanol were purchased from POCH, Poland.

Methods

Biomaterials preparation pathway

Gold nanoparticles were obtained in a reduction reaction of chloroauric acid. As a reducing agent orange peel extract was used which was prepared under microwave-as-

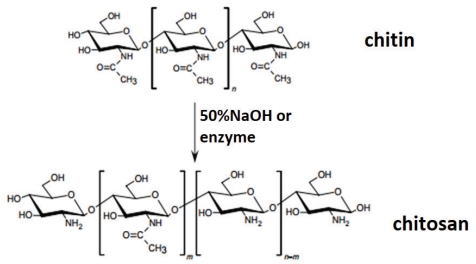

Fig. 1. Chitosan preparation
Rys. 1. Przygotowanie chitozanu

Tab. 1. Chitosan biomaterials synthesis parameters
Tab. 1. Parametry syntezy biomateriałów chitozanu

Sample	Chitosan, g	Acetone, ml		TEA, ml	Oleic acid chloride, ml	Deacetylation degree	Time, min	Temperature, °C
1	0.50	20		4.2	3	80	45	140
2						85		
3						90		
4						95		

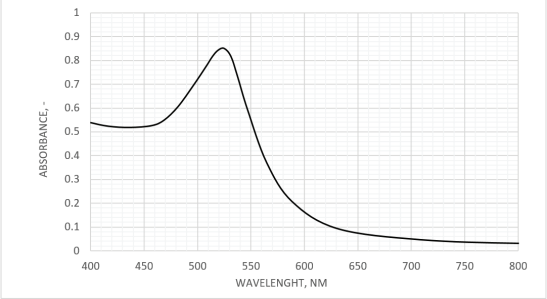

Fig. 2. UV-Vis spectrum of the prepared Au nanoparticles
Rys. 2. Widmo UV-Vis przygotowanych nanocząstek Au

sisted conditions. In brief, chopped orange peel fragments were placed in the Prolabo Synthwave microwave reactor for 5 min (80°C).

The extract was further purified on membrane filters. Chitosan acylation reaction was performed using as a raw material polymer of various deacetylation degree (80%, 85%, 90% and 95%) obtained from shrimps' exoskeletons. The acylation reaction was carried out using microwave irradiation. In the first step chitosan was activated using triethylamine dissolved in acetone. Then, acylation reaction was performed according to data presented in Table 1. To obtain 3D scaffolds, modified chitosan previously washed out with methanol and dried was swollen with 20 ml of the solution containing Au nanoparticles. Then, biomaterials were freeze-dried.

UV Vis spectroscopy analysis

UV-Vis spectrum was performed using Aligent 8453 spectrophotometer.

FT-IR analysis

FT-IR analysis was performed using IR Thermo Nicolet Nexus X 470 spectrometer (diamond crystal ATR), USA.

Biodegradation study

For the degradation study weighed samples were placed in the sterile SBF solution and their weight loss was determined after fixed time intervals according to the Equation 1. To verify their biodegradability, the samples were placed in the sterile SBF solution containing lysozyme – human enzyme which is present in various body fluids such as tears. The weight loss was determined using Equation 1:

$$(B)D = \frac{W_0 - W_t}{W_0} \cdot 100\% \qquad (1)$$

where:
$(B)D$ – (bio)degradation degree, %, W_0 – Initial weight of the analyzed sample, g, W_t – Sample weight after time = t, min

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

UV-Vis analysis

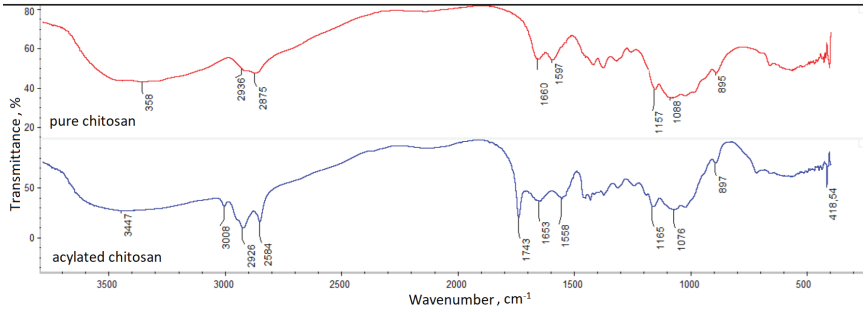

Fig. 3. FT-IR spectra of the prepared biomaterials
Rys. 3. Widma FT-IR przygotowanych biomateriałów

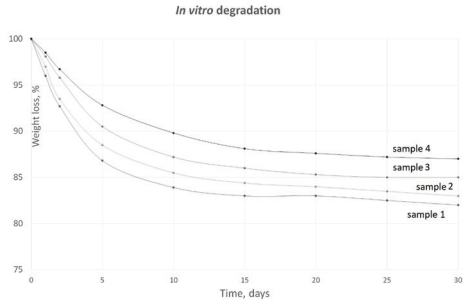

Fig. 4. Degradation study of the prepared biomaterials
Rys. 4. Badanie degradacji przygotowanych biomateriałów

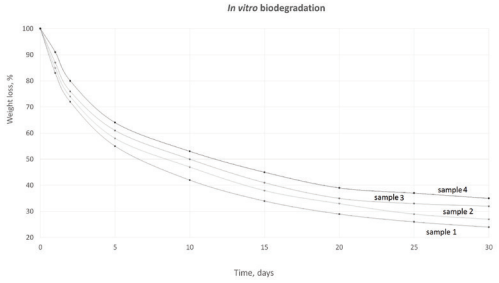

Fig. 5. Biodegradation study of the prepared biomaterials
Rys. 5. Badanie biodegradacji przygotowanych biomateriałów

Figure 2 presents UV-Vis spectrum of the prepared particles. It can be noticed, that the maximum absorption peak wavelength is typical for the Au NPs diameter of 20 nm. Therefore, it can be concluded that the nanoparticles were successfully obtained and their size is above 10 nm which will prevent their bioaccumulation and cytotoxicity.

FT-IR analysis

Figure 3 presents spectra of the pure chitosan and obtained samples. It can be noticed that unmodified chitosan spectrum shows some typical band coming from free hydroxyl groups at as well as acyl groups. Moreover, at the band corresponding to amide bonds present in acylated chitosan unit is visible as well as bands which are typical for free amino groups in deacetylated units. Additionally, band characteristic for glycosidic bonds as well as glucopyranose rings are noticeable. The other spectra show chitosan after acylation. It can be noticed that in the case of all samples some changes are visible. One may observe, that the intensity of amide bonds has significantly increased, whereas the intensity of free amino groups decreased. Moreover, bands coming from -CH- and -CH_2- are of much higher intensity which proves acyl chain incorporation. At the same time, bands coming from free hydroxyl groups as well as glycosidic bonds and pyranose rings are still presents which proves that no significant chitosan degradation has occurred.

Biodegradation study

Figure 4 presents results of in vitro degradation and biodegradation study carried out under human-like conditions. It can be noticed that all samples exhibited very good stability in the simulated body fluids since their weight loss in 30 days is up to 20%. On the other hand, one may observe that the biomaterials undergo biodegradation process in the presence of lysozyme which naturally occurs in human body and hydrolyses glycosidic bonds. Chitosan is a biodegradable poly-

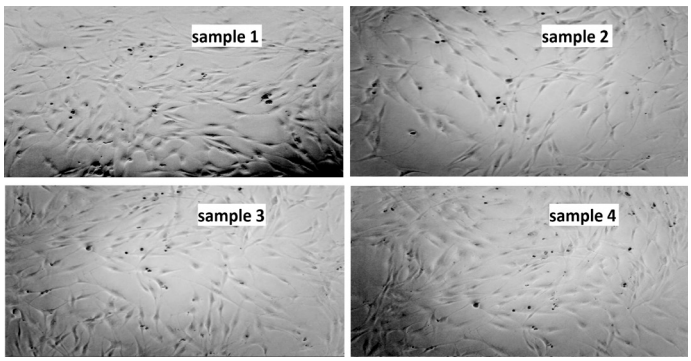

Fig. 6. Cytotoxicity of the prepared biomaterials
Rys. 6. Cytotoksyczność przygotowanych biomateriałów

mer, however chemical modification may hamper this property. The proposed strategy of bioactive scaffolds preparation enables obtainment of the biomaterials susceptible to natural degradation. It can be noticed (Figure 5) that all samples degradated in more than 60%. Thus, they can be considered as biodegradable and may be used under both in vitro and in vivo conditions since new tissue formation will be parallel with the materials degradation. The scaffolds will be naturally removed from the body and no reoperation will be needed which is very important for the patient.

Cytotoxicity study

The prepared biomaterials are dedicated to skin tissue engineering. The application of waste biomass may negatively affect the scaffolds cytocompability. The Figure 6 presents microphotographs (40x) of the human dermal fibroblasts after 48 hours of the study. It can be observed that the fibroblasts cultured in the presence of the biomaterials were flattened and of normal morphology without visible grains. Therefore, it can be stated that obtained scaffolds are biocompatible and provide appropriate environment for skin cells.

Conclusion

In this article a novel approach to chitosan was proposed. The scaffolds dedicated to full-thickness skin transplantation were prepared by acylation reaction using fatty acid derivative as modifying agent. The biomaterials were further modified with gold nanoparticles to enhance their bioactivity. The products were biodegradable under human-like conditions and had a positive impact on skin cells proliferation. Taken all together, the results confirmed preparation of novel biomaterials which can be successfully applied for damaged skin regeneration under in vitro conditions.

Acknowledgements

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Bioaktywne rusztowania do inżynierii tkanek skóry domieszkowane złotymi nanocząstkami przygotowanymi z biomasy odpadowej

Skóra jest pierwszą barierą przed patogenami i szkodliwymi czynnikami zewnętrznymi. Każde uszkodzenie tej tkanki może powodować zakażenie drobnoustrojami i zagrożenie dla narządów wewnętrznych. Oparzenia, które mogą być wynikiem narażenia na promieniowanie, chemikalia lub wysoką temperaturę, prowadzą do znacznego zakłócenia funkcji skóry. Najbardziej obiecującą metodą tego odzyskiwania tkanki jest medycyna regeneracyjna, która wymaga zastosowania trójwymiarowych biokompatybilnych rusztowań.Biomateriały umożliwiają namnażanie komórek skóry i tworzenie nowych tkanek w warunkach in vitro. Można je wytwarzać z polimerów syntetycznych i naturalnych oraz ich kombinacji. Zastosowanie dodatkowych składników, takich jak nanocząstki, może poprawić ich właściwości mechaniczne i mieć pozytywny wpływ na podziały fibroblastów i tworzenie się komórek. Jednym z najbardziej obiecujących surowców na rusztowania jest chitozan - pochodna chityny.Można go uzyskać z biomasy odpadowej, takiej jak egzoszkielety krabów, krewetek i homarów. Chitozan jest nietoksyczny, biodegradowalny i ma właściwości antybakteryjne.

Celem przedstawionych badań było uzyskanie nowych pochodnych chitozanu domieszkowanych nanocząstkami złota przy użyciu wyłącznie naturalnych składników, takich jak skórki pomarańczy i pochodna kwasu tłuszczowego. Proponowana strategia modyfikacji zaowocowała przygotowaniem nowego, biodegradowalnego i biokompatybilnego materiału o interesujących właściwościach. Produkty analizowano metodami UV-Vis i FT-IR. Rusztowania badano pod kątem ich podatności na degradację enzymatyczną. Na koniec biomateriały zweryfikowano pod kątem ich zgodności cytologicznej z ludzkimi fibroblastami skórnymi. Wyniki wykazały, że proponowany szlak syntezy zaowocował uzyskaniem biomateriałów chitozanu o wysokim potencjale w medycynie.

Słowa kluczowe: *odpady biomasy, nanocząstki złota, chitozan, biomateriały, Zielona Chemia*