CHITOSAN-BASED NANOCOMPOSITES AS POTENTIAL MATERIALS FOR NERVE REGENERATION

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Abstract

The nanocomposite material based on chitosan was obtained and characterized. Commercially produced biopolymer at 85% deacetylization degree was used. The biopolymer matrix was modified with carbon nanofillers such as graphite oxide (GO), carbon nanotubes (CNTs) and nanontubes with the surface affected by carboxyl groups (CNT-COOH). The obtained nanocomposites were formed by means of two methods: casting (to manufacture nanocomposite foils) and liofilization (to manufacture porous nanocomposite materials). Their electrical properties and microstructure were examined. The tests proved that adding the carbon nano-filler results in high resistivity (graphite foils, carbon nanotubes) and also the average size of pores in liofilized materials. Additionally, the electric potential of the materials may be improved by surface processing (EPD- electrophoretic deposition). The described materials are an alternative to polymer nerve implants e.g. tubes or hydrogels which are already present on the market and applied to regenerate nerves.

Keywords: *nanocomposites, chitosan, carbon fillers, guided nerve regeneration (GNR)*

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Introduction

Peripheral nervous system disorders (OUN) are a serious social problem, as approximately 2-5% of patients never fully recover from the damage and most of them (about 3%) are considered disabled. Both neurosurgery and neurology have proven that peripheral nerves display a potential for recovery. Biomaterials engineering focusing on designing specific materials may also facilitate the process of nerve regeneration. Guided nerve regeneration (GNR) means creating paths to direct the axons' growth. Such implants are most often manufactured from pure and modified polymers. Their objective is to provide the proper durability of the material in vivo conditions, efficient mechanical and functional properties, and such a microstructure that will facilitate the migration of growth factors and nutrients. Using degradable products means that a patient does not have to be reoperated on to remove the unnecessary material.

The in vivo durability of the material might be modified both by the type of the material and its porosity (the more porous material, the faster degradation). That is why the most popular polymers applied in GNR techniques are: polylactides (PLA), polyglicolides (PGA), their copolymers (e.g. PLGLA) and natural biopolymers such as chitosan (CS).

Another desirable feature is high electrical conductivity of the implant that will stimulate the regeneration of the damaged nerves. The conductivity phenomenon is present in every animal tissue. Unfortunately, it is often neglected in the process of designing nerve implants.

There are two methods to improve the electrical properties of the implant. One method involves the necessity to use piezoelectric polymers, such as PVDV (polydifluorovinylidene), PTFE (polytrifluoroethylene) or their copolymers, that are able to generate the surface charge under the influence of slight mechanical stresses. In the other method a certain number of carbon fillers is introduced into the polymer matrix in order to cross the percolation threshold and induce electrical conductivity of the material [1]. In the latter method carbon nanotubes (CNT), graphite (GR) and, as of late, graphene (G) are used. Since the volume modification is often not efficient enough, the electrophoretic deposition method (EPD) is used to increase the material's electrical conductivity. In this procedure carbon particles are introduced to improve electrical properties of the surface [2].

This paper presents the implant designed for guided nerve regeneration. The implant is made of two different materials: the outer chitosan membrane and the scaffolding filling. The chitosan membrane is modified in volume by means of CNT,GR,G, whereas its surface is modified with different carbon nanoforms (CNT). The porous scaffolding constitutes the filling of the membrane; its role is to facilitate the axial pathfinding.

In our work nanocomposite chitosan membranes were obtained by casting and their structure was modified by depositing the layer of carbon nanotubes (EPD). The porous scaffolding was obtained through liofilization, using various forms of carbon (GR, CNT) as fillers.

The microstructure of the porous background was examined using SEM imaging. It was also established how different types of modifiers affect the shape and size of pores. Electrical properties of the surface were tested both for initial nanocomposite chitosan membranes and the EPD-modified ones. The most advantageous material composition was selected, which is the scaffolding with the highest porosity and the most resistive membrane.

Materials and method

The base polymer was chitosan (CS) of viscosity 200-800cT and 75-85% degree of deacetylation (purchased from Sigma-Aldrich). The carbon fillers were: commercially available carbon nanotubes (purchased from NanoAmor, US), graphite oxide (AO-4, from Graphene Supermarket) and graphene (Graphene Supermarket). Producent date showed TABLE 1. The porous background with 2%wt filler was prepared in the shape of cylinders measuring 0.9x1cm. 3% acetic acid was used as a solvent. The membranes contained 1%wt of nano-filler. Both forms of the nanocomposite were air-dried. Then the materials were observed using a scanning miscroscope (Nova NanoSEM). The electrical properties of nanocomposite membranes were established with a multimeter (EAT 200). Both the volume-modified foils and the volume- and surface-modified foils were tested.

TABLE 1. Producent date of nanofillers.

Nanofillers	Shape and size of nanofillers	Specific surface area [m^2/g]
MWCNT (Nano Amor)	d=2-5 [nm], l=10-12 μm	450-560
MWCNT-COOH (NanoAmor)	d=0,7-2 [nm], l=15-30 μm	660
A6 (Graphen Supermarket)	d=6nm, a=2-3 μm	120
AO-4 (Graphene Supermarket)	d=60 nm, a=3-7 μm	<15

Results and discussion

Adding the nano-filler to the chitosan matrix changes the electrical potential of the material in a significant way. Using 1%wt of unmodified nanotubes set the material resistivity at the level of 350-78 kΩ, while the CNTs modified with carboxyl groups increased the value to 8-9 MΩ. It was established that additional surface modifications with different forms of carbon increase the resistivity of the system only if the modified material is carbon nanotubes. However, this change is rather slight, as compared to the initial material's value in the applied conditions (increase by 15-20% only). Meanwhile, the presence of fillers has a strong influence on the shape and size of pores in the liofilized systems. Introducing the carbon nanotubes results in elliptical and irregular pores. Introducing graphene and graphite oxide makes the pores more circular and homogenous (FIG. 1).

Conclusions

The proposed nanocomposite systems: active electrical foil and porous fulfilling nanocomposite material seem to meet the requirements for material used in guided nerve regeneration concerning damages in the peripheral nervous system.

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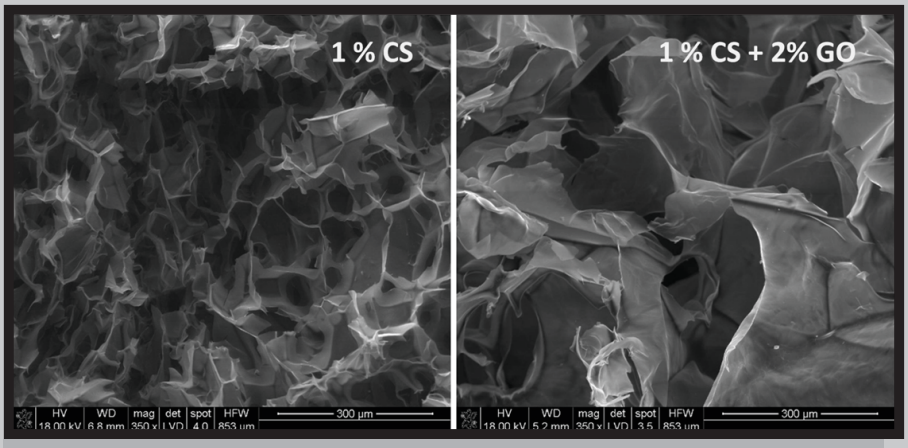

FIG. 1. Mictrostructure of liophilised chitosane and chitosane witf graphite.

COMPARISON OF DURABILITY OF RESORBABLE POLYMER PINS IN IN VITRO AND IN VIVO CONDITIONS. PRELIMINARY STUDY

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Abstract

The work focuses on polymeric cartilage/bone pins (pegs) that were manufactured and tested to assess their application in meniscus injuries. The bone pins measuring 1,2 and 4 mm in diameter were produced from certified resorbable PLDLA by means of hot pressing (at 126°C). In order to establish the material characteristics, tests of mechanical properties, structural testing and stability tests were performed in vitro (an immersion medium: water/ PBS buffer). It was established that after three months of incubation the initial implant's bending strength (120 MPa) decreased by 35%, whereas its tensile strength (52 Pa) weakened by 60%. The degree of degradation did not affect the pH of the immersion fluid. The observed physical changes of the implant, such as: the mass decrease, the change of shape, the increase of crystallinity (DSC/TG), the number of polymer terminal groups (-OH, -COOH), proved the advanced degradation process of PLDLA pins. Implants of particular behaviour were inoculated into the tibia of a New Zealand rabbit. In vivo tests were conducted to confirm the changes observed in vitro. Monitoring of the degradation process was performed after three months following the implantation by means of control X-ray and computed microtomography (μCT).

Keywords: *meniscus, bone pins, polylactide, regeneration*

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