PREPARATION AND CHARACTERIZATION OF A NEW GENERATION OF CHITOSAN HYDROGELS CONTAINING PYRIMIDINE RIBONUCLEOTIDES

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Abstract

Damage to the nervous system, in particular spinal cord injuries, is a burden for the patient and is usually the cause of irreversible disability. The progress observed in the last decade in the fields of biology, biomaterial engineering and neurosurgery has created new treatment solutions while preventing further neurodegenerative processes. The most important research is focused on the implementation of polymer structures in clinical practice, especially chitosan hydrogels, which are the scaffolds for regenerating axons. This article presents a new generation of biomaterials that have the ability to gel in response to temperature changes; they are intended for injectable scaffolds for nerve cell cultures. Two types of hydrogels were prepared based on chitosan lactate and chitosan chloride using uridine 5'-monophosphate disodium salt. The structure of the systems was observed under a scanning electron microscope and examined using Fourier transform infrared spectroscopy. In addition, thermal properties were tested using differential scanning calorimetry.

Keywords: *chitosan, biomaterial, hydrogel, neural tissue regeneration*

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1. Introduction

From the point of view of anatomy, the nervous system of vertebrates, including humans, can be divided into central and peripheral. The central nervous system (CNS) consists of the brain, cerebellum, brainstem and the spinal cord. Other elements of nerve tissue (even cranial, spinal nerves and ganglia) are classified as the peripheral nervous system (PNS). The CNS is the centre of coordination of all life processes of the body. Its main role is to receive, analyse and process the signals coming from the external and internal environment of the body, and then send them to the effector organs. On the other hand, the PNS may be seen as a complex network of ducts, accompanied by sensory receptors and clusters of nerve cells called ganglia. The main function of the PNS is the transmission of sensory and motor signals between body tissues and the CNS [1–3].

Damage in the CNS is currently a major problem both from a clinical and social point of view. Spinal cord injuries often lead to irreversible disability, and the methods of their treatment are limited and focused mainly on minimizing the effects of injury through the use of rehabilitation. Difficulties in convalescence result from damage to the neuronal cell bodies. In addition, there are cellular reactions leading to the formation of glial scars. For this reason, over the past decade, efforts have been made to identify innovative supportive to prevent further neurodegenerative processes within the spinal cord. The use of polymer structures constituting a scaffold for regenerating axons is particularly promising. Among the alternative therapeutic solutions, great emphasis is placed on the use of biodegradable polymers to avoid the need for reoperation in order to remove the implant after the nerve regeneration process is completed [4–6].

When considering the possibility of using biopolymers in the treatment of damage within the nervous system, it should be noted that the main current of research is focused on projects concerning hydrogels, including chitosan gels. They are characterized by high water content and favourable mechanical properties. In addition, their structure may contain drugs that can be delivered locally to promote the sealing of the damaged dura mater. The interest in chitosan hydrogels is also due to the fact that the phase transition from sol to gel occurs at the physiological temperature of the human body, which allows the scaffolds to be put into places that are hard to reach by injection [7–11].

Based on the literature on the subject, there are many possibilities for the production of thermosensitive chitosan hydrogels. These systems are mainly formed from low-salt solutions of chitosan salts using β-glycerophosphate disodium (Na-β-GP) as the neutralizing agent, polyvinyl alcohol or sodium bicarbonate. It is also possible to obtain hydrogels by means of an enzymatic reaction as a result of the presence of urea and urease in the solution of chitosan salts and on the basis of glucose-1-phosphate (G1-P) and glucose-6-phosphate (G6-P) compounds, as well as with a polyol-free phosphate salt (Na_2HPO_4). In addition, the biomaterials intended for injectable scaffolds for osteoblast cultures can be produced using an aqueous solution of calcium β-glycerophosphate [12–17].

This article presents a new form of chitosan gels with thermosensitive properties, which are intended for injectable scaffolds for nerve cell culture. These hydrogels are formed using uridine 5'-monophosphate (UMP) disodium salt. The systems are characterized by flexibility and softness, and their properties are similar to those of living tissues. The research was carried out to determine the characteristics of the obtained biomaterials.

The innovation of the developed chitosan system is their use as a substance enabling the phase transition of the sol into a gel of the pyrimidine ribonucleotide, which additionally has a regenerative effect on the components of the nervous system by improving neurotransmission. In the available literature, there is no information on the preparation of

thermosensitive chitosan gels with the participation of the UMP disodium salt. Only the production of hydrogels using derivatives of uridine (oUrd) and uridine monophosphate (oUMP) in combination with glutaraldehyde (AG) has been discussed, but these gels did not show phase transition under the influence of the temperature [18]. UMP is an organic chemical compound, a ribonucleotide that is part of RNA. It is an ester of phosphoric acid and uridine. It consists of the phosphate group, pentose sugar (ribose) and the nucleobase uracil (Fig. 1).

Nucleotides, including UMP, are often used in supplementation for the treatment of neurodegenerative diseases and in patients suffering from polyneuropathy. They have been proven effective in treating the cause of the myelin sheath lesion. In addition, UMP is used in the human body to produce phosphatidylcholine, which is the basic component of the lipid bilayer of biological membranes found in the body, including nerve cell membranes [19, 20].

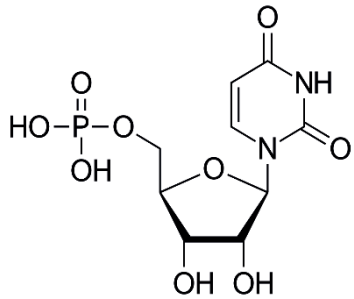

Figure 1. Structural formula of uridine 5'-monophosphate.

2. Materials and Methods

2.1. Preparation of Chitosan Hydrogels

The samples were prepared with the use of two acid solvents: lactic acid (the CH/LA/UMP system) and hydrochloric acid (the CH/HCL/UMP system). Chitosan lactate/chloride solutions were prepared by swelling 0.4 g of chitosan (CH, from crab shells with low viscosity and a degree of deacetylation ~79.5%; Sigma-Aldrich, product no. 50494-100G-F) in 16 mL of 0.1 M $C_3H_6O_3$ (LA, Sigma-Aldrich, product no. L6661-100ML) or 0.1 M HCl (Sigma-Aldrich, product no. H1758-100ML). The solutions were stirred until complete dissolution and left at room temperature for 24 h. A cooled solution of UMP disodium salt (Sigma-Aldrich, product no. U6375-5G) was then added drop by drop. The UMP disodium salt solution was obtained by dissolving 2 g UMP in 2.5 mL distilled water at 4°C. The resulting samples were incubated at 37°C in order to complete their gelation. The obtained hydrogels (in the form of cylinders) were frozen and then lyophilized (Fig. 2).

2.2. Methods

The properties of chitosan hydrogels after their lyophilization were studied. The morphology of scaffolds was analysed using a scanning electron microscope (SEM; FEI, Quanta 200F, Hillsboro, OR, United States).

The structural characteristics were based on the analysis of Fourier transform infrared spectroscopy (FTIR). The FTIR spectra were obtained using a Nicolet™ iS™10 FT-IR apparatus equipped with a monolithic diamond ATR crystal (Thermo Fisher Scientific Inc., United States), in the range 4000–500 cm^{-1}, with a resolution of 4 cm^{-1} and 100 scans.

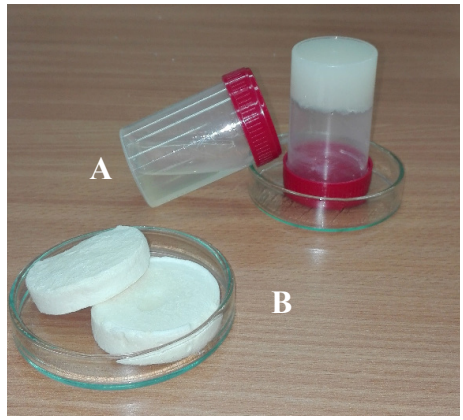

Figure 2. Thermosensitive chitosan hydrogels: (A) chitosan solution before (left) and after (right) gelation and (B) scaffolds after lyophilization.

Thermal analysis of hydrogels was carried out with a differential scanning calorimeter (DSC; FP90 Central Processor Mettler Toledo) at a heating rate of 10°C·min^{-1}. All samples were heated from 0 to 180°C; scaffolds were then cooled at a rate of 20°C·min^{-1}.

3. Results and Discussion

SEM is a valuable tool for evaluating the scaffold characteristics (morphology and pore size) of biomaterials and biological systems. The SEM images of chitosan thermosensitive gels after lyophilisation are shown in Fig. 3A (the CH/LA/UMP system) Fig. and 3B (the CH/HCL/UMP system).

The micrographs show that the developed hydrogels containing UMP disodium salt have a highly porous, homogeneous and interconnected structure. Both gel systems (CH/LA/UMP and CH/HCL/UMP) display similar spatial architecture. There are two pore sizes; most are approximately 2.5–3.0 μm, but there are numerous smaller pores, whose presence should positively affect the growth and proliferation of nerve cells. In summary, the lyophilization technique has proven to be a great method for the fixation of scaffolds for SEM studies because this technique clearly distinguishes the pores.

Fig. 4. displays the FTIR spectra of the obtained biomaterials. In the chitosan spectrum, in the range of wave numbers 3600–3100 cm^{-1}, there is an asymmetric wide band. The asymmetric shape of the peak, which is visible in the range of lower wave numbers, indicates the presence of strong hydroxyl bonds and the amine N-H groups in the structure. In the range of 2950-2850 cm^{-1}, the spectrum comprises two overlapping bands, which characterize the stretching vibrations in the aliphatic groups (-CH_2 and -CH_3). In addition, the FTIR spectrum shows a band at 1660 cm^{-1} (C=O stretching in the primary amide) and 1513 cm^{-1} (–NH_2 bending in the secondary amide); peaks with frequencies at 1420, 1375, 1315 and 1260 cm^{-1} (associated with oscillations characteristic of O-H and bending of C-H of CH_2 groups and representing C-O stretching of the primary alcoholic group -CH_2-OH [bending of -CH_2]); and bands representing the bridge: 1151 cm^{-1} (stretching C-O-C) and 1062 cm^{-1} (stretching C-O).

The following features characterized the spectra of chitosan hydrogels with the UMP disodium salt: a wide band at 3200 cm^{-1} (O-H bond); a peak at 2850 cm^{-1} (the aliphatic group CH_2); 1710 cm^{-1} (C_2=O stretching); and several peaks at 1490 cm^{-1} (bending N-H), 1390 cm^{-1} (CH_2 twist, in-plane deformation mode of N_3-H, deformation mode

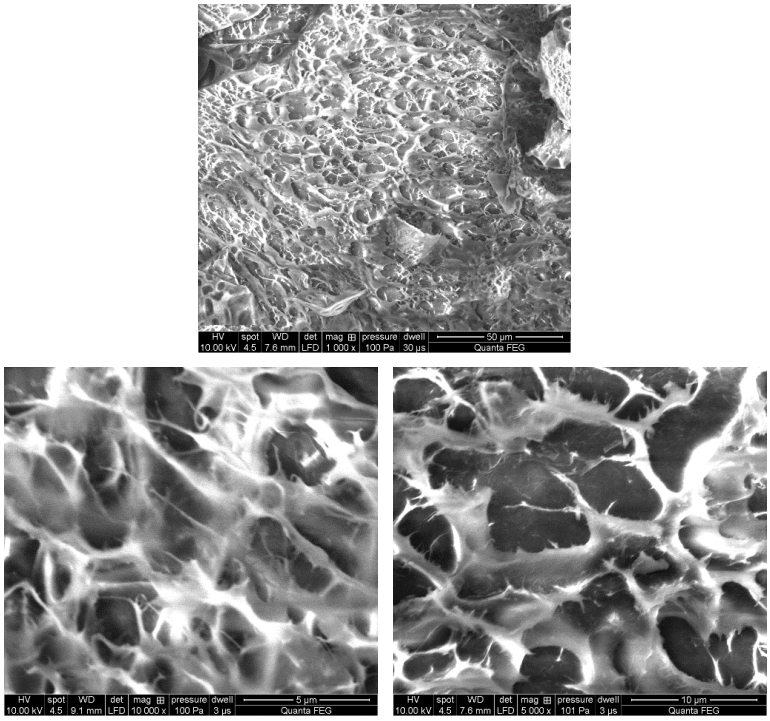

Figure 3A Scanning electron micrographs of the chitosan/lactic acid/uridine 5'-monophosphate system.

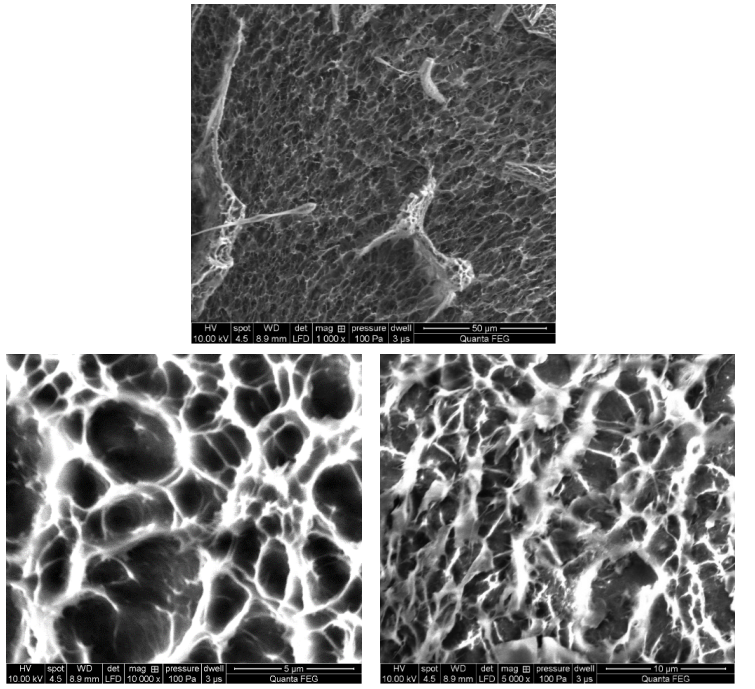

Figure 3B Scanning electron micrographs of the chitosan/lactic acid/uridine 5'-monophosphate system

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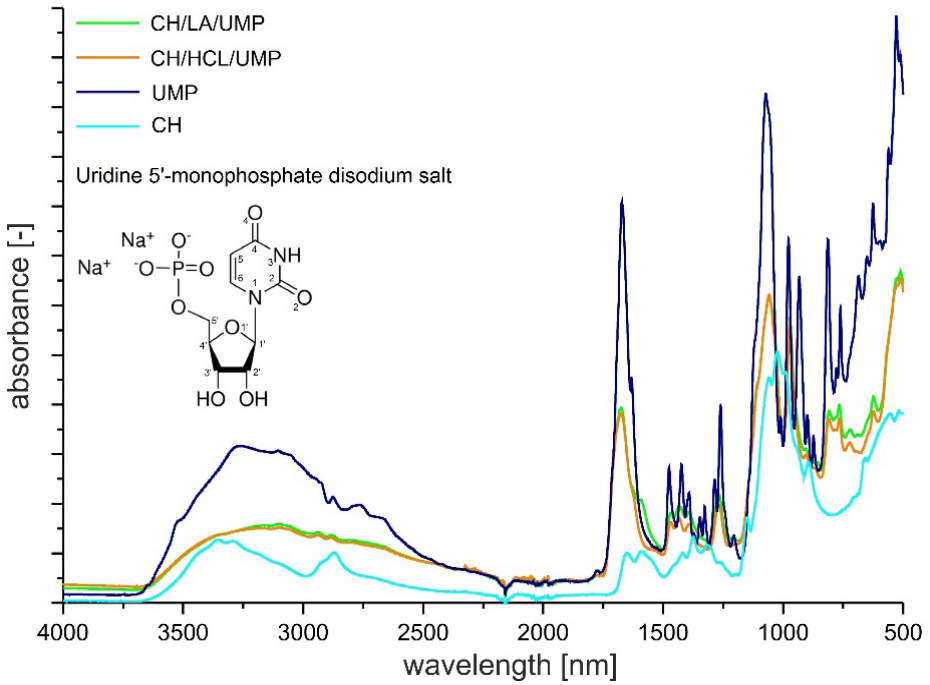

Figure 4. The Fourier transform infrared spectra of chitosan (CS), uridine 5'-monophosphate (UMP) disodium salt and chitosan salts after gelation. Abbreviations: HCl, hydrochloric acid; LA, lactic acid.

of O-H [ribose]), 1250 cm^{-1} (stretching mode of the N_1-C_2-N_3 ring, bending of C-H in uracil), 1100 cm^{-1} (ribose-phosphate, stretching mode of uracil ring), 1050 cm^{-1} (C-C stretching in ribose, C-O stretching in ribose, ring bending mode, ring stretching mode), 970 cm^{-1} (symmetric stretching of PO_3^{2-}) and 900 cm^{-1} (C-C stretching in ribose).

The bands for wave numbers 800, 750 and 700 cm^{-1} (P-O stretching, C-C stretching in ribose, C-H rocking in uracil, C=O rocking, C-C-O bending in ribose and bending mode of the uracil ring uracil) present in the UMP disodium salt spectrum disappear in the gel spectra. Interpretation of the FTIR spectra was based on previous studies [21, 22].

Fig. 5. presents the differential scanning calorimetry spectra. DSC spectra of hydrogels made from both chitosan lactate (the CH/LA/UMP system) and chitosan chloride (the CH/HCL/UMP system) are similar. The spectra have two bandwidths: a small peak at 56.4°C for CH/LA/UMP, 57.0°C for CH/HCL/UMP and a wide peak at 117.9°C for CH/LA/UMP or 111.5°C for CH/HCL/UMP. The bands of gels made from chitosan lactate show a shift towards higher temperatures. The wide bands are most likely related to the evaporation enthalpy of the water interface located in the pores of the gel. The enthalpy for the CH/LA/UMP system is 164.40 J/g, and 165.15 J/g for the CH/HCL/UMP. High temperatures for wide bands also indicate the presence of bound water in the structure of the hydrogels.

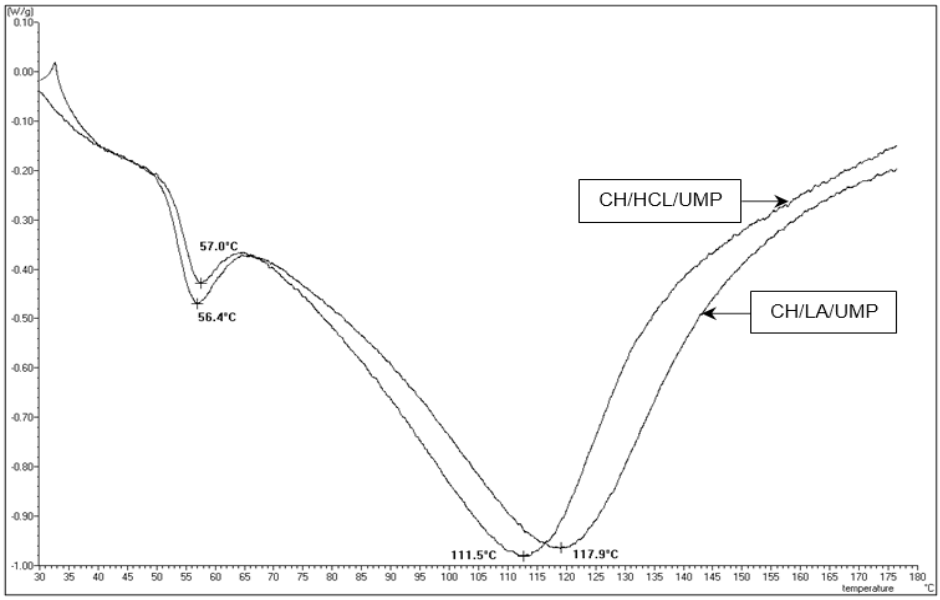

Figure 5. The differential scanning calorimetry spectra of chitosan (CS) hydrogels. Abbreviations: HCl, hydrochloric acid; LA, lactic acid; uridine 5'-monophosphate (UMP)

4. Conclusion

Thanks to the use of the nucleotide UMP, the prepared chitosan systems (CH/LA/UMP) and (CH/HCL/UMP) exhibit the features of thermogels, for which the temperature increase initiates the sol-gel phase transformation. The presence of UMP in the hydrogel structure gives them a wide range of applications in tissue engineering (especially in nerve regeneration). The chosen direction of research is innovative and extremely promising. Further experiments in this field will be conducted in the future.

5. Acknowledgements

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