BIOPOLYMER MICROSPHERES MODIFIED WITH MAGNESIUM AND ZINC IONS FOR TISSUE ENGINEERING APPLICATIONS

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Introduction

One of the problems still faced by various tissue engineering scaffolds is the lack of proper vasculature that would aid regeneration of tissue defects. Formation of a network of blood vessels through angiogenesis is one of the most effective ways to sustain healthy cells metabolism by delivering nutrients, signalling factors and removing waste. This is fundamental for successful tissue regeneration. Among many proangiogenic factors, metal ions, such as magnesium, strontium, copper, or zinc are particularly interesting due to their additional therapeutic activities [1]. They can be used in various systems. However, biopolymer-based carriers seem to be one of the most versatile and possess all the advantages of being naturally-derived, e.g. biocompatibility, sustainability, high availability, relatively low cost.
The aim of the study was to design and characterize polysaccharide-based systems for future use for angiogenesis enhancement in tissue engineering applications. Magnesium and zinc ions were selected as active molecules for surface modification of chitosan microspheres.

Materials and Methods

Chitosan microspheres were fabricated by dropping chitosan solution (1.5% CS, Chitosan 85/1500, Heppe Medical Chitosan GmbH (HMC+) in 1% acetic acid) into a constantly stirred gelling bath (5% sodium tripolyphosphate (TPP), 0.15M NaCl, 2% Span80) through a 0.7 mm needle. A volume ratio of CS solution to the gelling solution was set at 1:10. The formed beads were crosslinked for 48 h, subsequently centrifuged and washed with isopropanol, ethanol, and distilled water. To immobilize magnesium and zinc ions on the surface of CS carriers, microspheres were immersed in an appropriate ion solution (500 mg/L of Mg^{2+} or Zn^{2+}) for 48h under constant stirring, drained and washed with distilled water. Prior to characterization, all CS-based beads were frozen and freeze-dried. Microstructure (digital microscopy, SEM-EDS), chemical stability (weight loss, water absorption), structure (FTIR) and ion release (AAS - Atomic Absorption Spectroscopy, air-acetylane flame) were evaluated.

Results and Discussion

The parameters of the fabrication method were carefully optimized considering concentration of polymer solution, needle diameter, composition of a gelling solution, crosslinking time, etc. All of the above affect the final properties of the microspheres. The obtained chitosan beads were round and homogenous with a diameter of 904±94 μm (FIG. 1). After modification with Mg^{2+} and Zn^{2+}, slight morphology changes were observed as a result of exposure of hydrogel spheres to water-based ion solution. Also, the diameters increased to 1080±125 μm and 1138±79 μm for CS-Mg and CS-Zn, respectively. The presence of magnesium and zinc on the surface of the microspheres was verified by the SEM-EDS analysis.

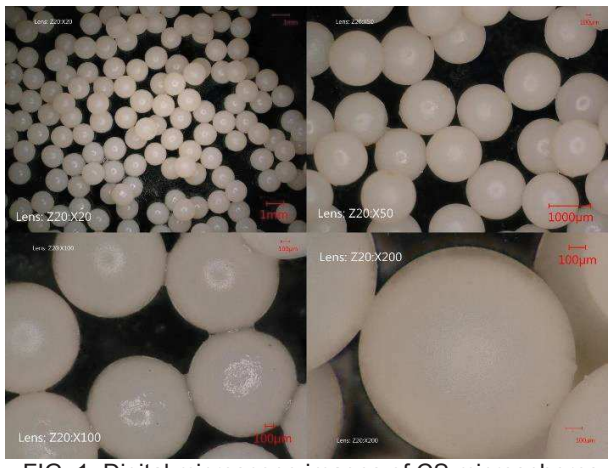

FIG. 1. Digital microscope images of CS microspheres (mag: x20, x50, x100, x200).

Chitosan was crosslinked in the TPP-based solution. FTIR spectra (FIG. 2) confirmed that phosphate groups were present in the final material. As expected, the crosslinking effect occurred due to the interactions between negatively charged PO^- groups of TPP and positively charged NH_3^+ groups of CS. Changes in the spectra related to Mg^{2+} and Zn^{2+} modifications were also visible.

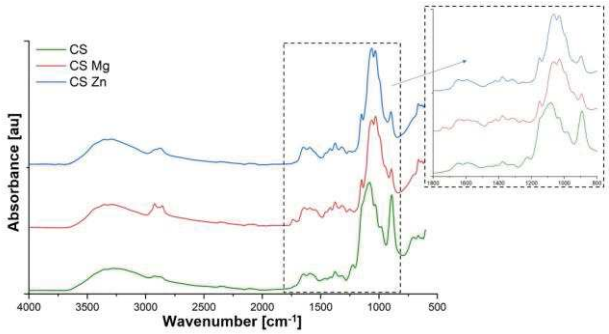

FIG. 2. Attenuated Total Reflection-Fourier Transform Infrared Spectroscopy spectra of unmodified (CS) and ion-modified samples (CS-Mg and CS-Zn).

AAS analysis showed that release kinetics differed for both ions. Higher final concentration was achieved for magnesium-modified CS beads. The release rate and kinetics can be modified.

Conclusions

Chitosan microspheres can be used as carriers of magnesium and zinc ions, known for their proangiogenic activity. Further, detailed studies are ongoing.

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References

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