Dual-Functional Polysaccharide Microgels Modulated by Therapeutic Metal Ions

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INTRODUCTION

In recent years, chitosan-based materials modified by therapeutic metal ions gained much attention in regenerative medicine and tissue engineering due to the synergy of polymer's biocompatibility and bioactivity of metal ions. Chitosan is a biocompatible, biodegradable and non-toxic cationic polysaccharide[1] whose properties can be modulated by complexation with therapeutic metal ions such as cupric (Cu^{2+}) or zinc (Zn^{2+}) ions.[2,3] Cu^{2+} ions are well known for stimulating the proliferation and differentiation of human endothelial and mesenchymal stem cells, while Zn^{2+} ions promote bone formation by enhancing osteoblast differentiation.[4] Furthermore, the complexation interactions between Cu^{2+} or Zn^{2+} ions with amino and hydroxyl groups,[5] leads to stable complex hydrogels. Combining biologically active ions with pH-responsive chitosan microspheres could generate multifunctional carriers and delivery systems. Still, there is no work focused on synthesis of Cu^{2+}/Zn^{2+}-chitosan complexes as microgels, i.e. in the form of microspheres. The aim of this work was to prepare and investigate the composition, morphology and cytotoxicity of Cu^{2+}/Zn^{2+}-chitosan complexes and their ability to form microgels using electrohydrodynamic atomization process.

EXPERIMENTAL METHODS

Chitosan (CHT) with the degree of deacetylation of 83.2 % (Chitoscience Chitosan 85/200, Heppe Medical Chitosan GmbH) was used. Zinc acetate dihydrate and copper acetate monohydrate were used as precursors for Zn^{2+} and Cu^{2+} ions, respectively. The Cu^{2+}/Zn^{2+}-chitosan hydrogels were prepared by mixing 1 wt.% CHT solution with metal precursor solutions, followed by gelation with 5 wt.% NaOH solution. The final concentration of Cu^{2+} and Zn^{2+} ions in prepared complex solutions was ranging from 0 to 5 mmol/L. Prepared bimetallic-chitosan complex solutions were denoted as Cu5/Zn0-CHT Cu3/Zn2-CHT, Cu1/Zn4-CHT and Cu0/Zn5-CHT. Obtained hydrogels were extensively washed with distilled water, frozen and lyophilized to obtain dry materials which were characterized by ATR-FTIR spectroscopy and XRD analysis. The samples' morphology and elemental analysis were investigated by scanning electron microscopy (SEM) with energy-dispersive X-ray spectroscopy (EDS), while their cytotoxic activity was evaluated on HEK293 cells by MTT assay for 72 hours. To obtain materials in the form of microgels, the electrohydrodynamic process was conducted at solution flow rate of 5 mL/h, the voltage of 22.5 kV and needle tip-to-collector distance of 10 cm. After neutralization in NaOH, microspheres were washed and dried in acetone.

RESULTS AND DISCUSSION

Bimetallic-chitosan hydrogels were successfully prepared by gelation of Cu/Zn-chitosan complex solutions. Visual assessment of hydrogels in their native wet state indicated stable structure of Cu5/Zn0-CHT Cu3/Zn2-CHT and Cu1/Zn4-CHT hydrogels, while Cu0/Zn5-CHT hydrogel was disintegrated during the washing step. The addition of Zn^{2+} ions decreased the stability of hydrogels which imply that Cu^{2+} ions are mostly responsible for stronger complexation reactions with functional groups of chitosan's chains. Such observation was confirmed by FTIR spectra which indicated physical interactions between metal ions and polymer. Furthermore, X-ray diffraction patterns of dry materials showed the changes in the intensity and width of diffraction maximum characteristic for chitosan in $c(Cu^{2+})$-depended manner indicating the changes in chitosan crystallinity caused by cupric ions. Furthermore, X-ray diffraction patterns did not show any presence of Cu or Zn species. The MTT assay indicated cytotoxic activity at higher copper concentration, while the addition of zinc ions resulted in good cytocompatibility. Prepared Cu/Zn-CHT complex solutions were then successfully transformed into spherical microgels by electrohydrodynamic atomization resulting in stable microspheres with average diameter of 65 – 95 μm.

CONCLUSION

In this work, stable complex hydrogels based on chitosan with two different therapeutic metal ions were successfully prepared. Obtained Cu/Zn-CHT materials showed porous microstructure without formation of Cu or Zn inorganic phase. The MTT assay indicated that the cytotoxicity of materials can be modulated by the addition of Zn^{2+} ions. Furthermore, prepared Cu/Zn-chitosan complexes could be promising systems for microspheres production as potential bioactive carriers.

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