Development and evaluation of a chitosan based injectable gel for brain cancer treatment

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Statement of Purpose: Recently, thermo-sensitive injectable systems have been studied to carry therapeutic agents, cells or molecules for local delivery. In particular, chitosan based injectable gels have notable potential as a carrier to deliver specific drugs at target area, exhibit controlled drug release, and increase drug loading rate for a wide range of biomedical applications. The β-glycerophosphate (β-GP) has attracted a lot of attention in forming thermo-sensitive chitosan based gel delivery vehicles [1]. The objective of this study was to develop and characterize a heat induced chitosan/β-GP gel and evaluate its anti-tumor effect on cancer cells (human U87 glioblastomas and rat C6 glioma cells) when loaded with ellagic acid *in vitro*. In our previous study [2], chitosan/ellagic acid (Ch/EA) composite films inhibited proliferation of human WM115 melanoma, human U87 glioblastoma, and rat C6 glioma cells *in vitro* and significantly inhibited rat C6 glioma growth *in vivo*. This study has focused on thermal gelation at body temperature and biocompatibility of the chitosan gel system and delivery of ellagic acid by simple injection for cancer treatment.

Methods: Chitosan (≥ 310 KDa, ≥75% DDA) based injectable solutions were prepared with various concentrations of β-glycerophosphate disodium salt (β-GP) and loaded with ellagic acid to evaluate anti-tumor effect on brain cancer cells. We characterized the properties of the chitosan/ β-GP gels with regard to chemical structure, surface morphology, and viscoelasticity using FTIR, SEM, rheological analysis, and gelation time determination. *In vitro* release study was performed by using a UV spectrophotometer, and enzymatic degradation rate was determined by analyzing the increased free amino groups. We also investigated cytotoxicity of the chitosan/ β-GP system as well as anti-tumor effect on cancer cells via indirect cell culture by MTS assay.

Results/Discussion: FTIR spectra shows that the protonation of phosphate groups in the Ch/β-GP solution occurred with increasing temperature as shown in Figure 1. The diprotonated phosphate ($-H_2PO_4$) groups appeared in the Ch/β-GP at 37°C, suggesting the nonprotonated phosphate ($-PO_4^{2-}$) ions accepted protons (H^+) with increasing temperature. As a result, there is a decrease in the protonation around the chitosan molecules, indicating decreased solubility of chitosan molecules. This result demonstrated that the chitosan/β-GP solution induced thermal gelation around body temperature via hydrophobic interaction between chitosan molecules. Gelation temperature was decreased with increasing final pH of the gel forming solution. The gelation time decreases exponentially with increasing temperature. The chitosan/β-GP gels were enzymatically degradable, and the release of ellagic acid from the chitosan/β-GP gels was accelerated via diffusion by pores of gel network as well as polymer matrix degradation. A dialysis of an acidic chitosan solution reduced the concentration of β-GP to reach around the pH 7.2, resulting in improved biocompatibility. Cells encapsulated inside gels were viable, but in their round shape. After they reached the rigid flat surface, the cells were able to spread, grow, and proliferate. The chitosan/β-GP gels loaded with ellagic acid induced significant decrease in the MTS activity for human U87 glioblastoma and rat C6 glioma cells in an ellagic acid concentration-dependent manner.

Conclusions: The chitosan/β-GP solution formed heat-induced gel network at body temperature, and the gelation temperature and time were affected by the final pH of the chitosan/β-GP solution. The ellagic acid was released from the chitosan/β-GP gels via diffusion by pores of gel network as well as polymer matrix degradation. A dialyzed chitosan solution reduced the amount of β-GP needed for gelation and improved biocompatibility. The chitosan/β-GP gels loaded with ellagic acid inhibited the cancer cell growth in an ellagic acid concentration-dependent manner.

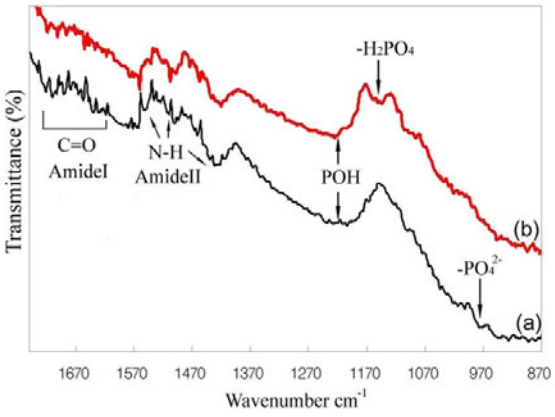

Figure 1. FTIR spectra of the chitosan/β-GP solution: (a) at 5°C; (b) 37°C. The chitosan solution contained 0.088 M of β-GP solution and its final pH was 7.23.

References:

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2. Kim S, Gaber MW, Zawaski JA, Zhang F, Richardson M, Zhang XA, Yang Y. Biomaterials 2009; 30(27): 4743-4751.