Development of Injectable Thermogelling Chitosan-Inorganic Phosphate Solution for Biomedical Applications

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Statement of Purpose: Injectable in situ gelling hydrogels have tremendous potential as ideal carrier matrices for a wide range of biomedical and pharmaceutical applications such as drug delivery and tissue engineering.[1] The advantages of injectable gelling systems over preformed matrices include introduction into the body in a minimally invasive manner, the ability to provide a good fit, and delivery of bioactive molecules or cells to the defect site under mild conditions.[1] For effective clinical application, injectable systems should be able to gel at mild physiological conditions in a clinically relevant time period with minimal syneresis. The present study deals with the development of a novel injectable, neutral, physiological temperature gelling composition from a biocompatible natural polymer 'chitosan' and an inorganic phosphate salt 'ammonium hydrogen phosphate (AHP)'. The feasibility of using the gels as a cell carrier matrix has also been demonstrated.

Methods: Chitosan from crab shells (minimum 85% deacetylation) and ammonium hydrogen phosphate (AHP), were procured from Sigma (St Louis, MO). Acetic acid (0.5%) solution of chitosan (~2.8%), pH~ 5.6 was prepared and stored at 4°C. For gelling studies chitosan (5 mL) was stirred magnetically in an ice bath. An appropriate amount of AHP was added to the chilled solution. The gelling time was determined by incubating the solution at 37°C. The flowability of the solution was checked every 30 sec for 30 min by tilting the vial. The time at which the solution stopped flowing was taken as the gelling time (n=4). The effect of diluents on the gelling time was determined by adding different volumes of diluents [water (pH 7.0), phosphate buffered saline (PBS) and minimum essential media MEM)] to 5 mL of the gelling solution followed by gelling time determination. For cell encapsulation, MC3T3-E1 cells were encapsulated in sterile chitosan-AHP solution and cultured in MEM. The viability of encapsulated cells after 14 days in culture was determined by the LIVE-DEAD cytotoxicity assay.

Results / Discussion: The feasibility of developing a thermogelling chitosan solution in the presence of AHP was demonstrated (Figure 1). Figure 2 shows the gelling time of chitosan-AHP solution as a function of the amount of AHP added. It has been found that addition of basic AHP solution (pH~8.6) to chitosan solution increased the pH of the chitosan solution without spontaneous gelation. (~7.00 to 7.2). Unlike multivalent phosphates, such as tripolyphosphate, which is known to form pure ionic crosslinking with chitosan, the divalent phosphate ions from AHP did not induce a pure ionic cross-linking. Thus the addition of AHP salt to chitosan solution is presumably modifying the hydrophobic and hydrogen bonding interactions between chitosan chains and the electrostatic interactions between the chitosan chain and the divalent phosphate ion. This is evident from the stability of chitosan-AHP solution at higher pHs

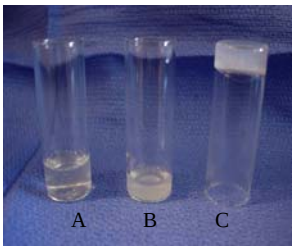

Figure 1. Thermogelation of chitosan-AHP. A. Chitosan solution B. Chitosan-AHP solution, C. Thermogelled chitosan-AHP

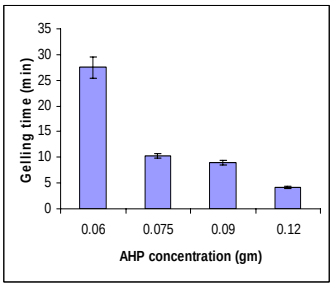

Figure 2. Effect of AHP concentration on gelling time

Diluent (AHP-0.075 g)	Gelling time (min)
2 mL, PBS	10.6 ± 0.5
5 mL PBS	11.8 ± 0.5
2 mL MEM	11.5 ± 0.4
2 mL water	10.8 ± 0.6

Table 1. Effect of diluents on the gelling time

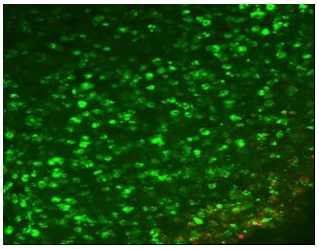

Figure 3. Viability of MC3T3-E1 cells in chitosan-AHP gel

(pH 7 - 7.2) and the temperature controlled gelation process of the system. The concentration of AHP (Figure 2) has a significant effect on the gelling time of the chitosan-AHP solution which shows the effect of electrostatic interaction. Since the thermogelling injectable system has the potential to be used as carrier agents for cells or bioactive molecules, the effect of the addition of various diluents on the gelling time of chitosan-AHP solution was evaluated. Table 1 shows the effect of diluents on the gelling time of chitosan-AHP solution. The table shows that dilution of chitosan–AHP using various diluent solutions did not significantly affect the gelling time of the solution. Figure 3 shows the confocal fluorescence image of cells encapsulated within chitosan-AHP gels. It can be seen that the cells were homogeneously distributed within the gels and almost all the cells remained viable after 14 days in culture.

Conclusions: An injectable thermogelling solution was developed by the addition of AHP to chitosan solution. The addition of AHP to chitosan resulted in the formation of near neutral solution that gelled in a clinically relevant time period when incubated at 37°C. The encapsulation study performed using MC3T3-E1 demonstrated the potential of the chitosan-AHP system as a cell carrier matrix. These experiments collectively indicate the potential of chitosan-AHP as an injectable carrier vehicle for a variety of biomedical applications.

References: . Ruel-Gariepy E, Leroux JC. European J Pharma Biopharma 2004; 58: 409-26