ACCELERATION OF GELATION AND PROMOTION OF MINERALIZATION OF CHITOSAN HYDROGELS BY ALKALINE PHOSPHATASE

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Abstract

Thermosensitive chitosan hydrogels containing sodium beta-glycerophosphate (β-GP), whose gelation is induced by increasing temperature to body temperature, were functionalized by incorporation of Alkaline Phosphatase (ALP), an enzyme involved in mineralization of bone. ALP incorporation led to acceleration of gelation upon increase of temperature for four different chitosan preparations of differing molecular weight, as demonstrated by rheometric time sweeps at 37ºC. Hydrogels containing ALP were subsequently incubated in calcium glycerophosphate (Ca-GP) solution to induce their mineralization with calcium phosphate (CaP) in order to improve their suitability as materials for bone replacement. Incorporated ALP retained its bioactivity and induced formation of CaP mineral, as confirmed by SEM, FTIR, Raman spectroscopy, XRD, ICP-OES, and increases in dry mass percentage, which rose with increasing ALP concentration and incubation time in Ca-GP solution. The results demonstrate that ALP accelerates formation of thermosensitive chitosan/β-GP hydrogels and induces their mineralization with CaP, which paves the way for applications as injectable bone replacement materials.

Keywords: *chitosan, thermosensitivity, mineralization, composite*

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Introduction

Chitosan, a polysaccharide derived by deacetylation of chitin, is biocompatible, biodegradable , non-toxic, non-immunogenic, and possesses antibacterial properties. Chitosan hydrogels can be formed by neutralization of an acidic chitosan solution by addition of sodium beta-glycerophosphate (β-GP) [1]. These hydrogels have the added advantage of thermosensitivity, i.e. gelation can be induced by increasing temperature to the range 30-60 °C, and allow incorporation of bioactive substances such as enzymes, including alkaline phosphatase (ALP), the enzyme responsible for mineralization of bone. With respect to bone substitution, the presence of a ceramic phase based on calcium phosphate (CaP) leads to a number of advantages, including increased bioactivity (formation of chemical bonds with surrounding bone after implantation) and affinity for biologically active proteins such as growth factors [2]. The strategy of ALP incorporation followed by incubation in a solution containing calcium ions and glycerophosphate (GP) appears to be applicable to a wide range of hydrogels [3]. Both calcium ions and glycerophosphate diffuse into the gels containing ALP. ALP cleaves phosphate ions from glycerophosphate, which are then free to react with calcium ions to form insoluble CaP which precipitates and remains trapped within the gel. The current study aimed to investigate the effect of ALP incorporation on gelation speed. The amount and nature of mineral formed was also investigated.

Materials and methods

Chitosan hydrogels were produced according to a protocol based on that of Chenite et al. [2] Briefly, 0.4 g chitosan was dissolved in 16ml 0.1M HCl. 10 g sodium glycerophosphate were dissolved in 10ml water. ALP was dissolved in water at concentrations of 0 and 2.5mg/ml. 3.6 ml chitosan solution was mixed with 0.4 ml Na-GP solution and 0.4 ml ALP solution, to yield a chitosan concentration of 20.5 mg/ml, a Na-β-GP concentration of 90.9 mg/ml and ALP concentrations of 0 and 0.23 mg/ml. Gelation took place at 37ºC overnight. Mineralization was induced by incubation in 0.1M calcium glycerophosphate (Ca-GP). Prior to analysis, hydrogels were rinsed three times with Milli-Q water, then incubated in MilliQ water for 24h with the aim of removing residual Ca-GP. For rheological studies, gel components were mixed in the same proportions, but with a total volume of 0.5 ml. Rheological measurements were carried out on an AR2000 rheometer, TA instruments, using a 20 mm flat plate geometry and a gap of 0.7mm. Time sweeps were performed at 37ºC at an oscillatory stress of 5 Pa and frequency of 1Hz. The gelation point was defined as the point where the storage modulus (G') exceeded the value of the loss modulus (G'') (Figure a,b). For each chitosan preparation and ALP concentration, measurements were performed at least 3 times. The dry mass percentage, i.e. the gel weight percentage not consisting of water, was calculated as: (weight after incubation and subsequent freeze-drying/ weight after incubation but before freeze-drying)*100. This served as a measure of the extent of mineral formation. Formation of CaP was demonstrated by FTIR, Raman, XRD, SEM and ICP-OES.

Results and discussions

Addition of ALP accelerated gelation of all four chitosan preparations in this study (FIGURE a,b,c). Gelation times of all four chitosan preparations tested were were similar in the absence of ALP and shortened by ALP addition (FIGURE c), which demonstrates the feasibility of ALP-accelerated gelation for different preparations. Mineralization of chitosan hydrogels by ALP was demonstrated by an increase in dry mass percentage with increasing ALP concentration, changes in morphology of chitosan and appearance of mineral-

ized deposits by SEM (Figure d,e). Further evidence was provided by the presence of XRD peaks characteristic for apatite (FIGURE f) and bands typical for CaP in FTIR and Raman spectra (FIGURE g,h), and ICP-OES detection of increases in mass percentage attributable to Ca and P as well as Ca/P molar ratio in hydrogels containing ALP.

Conclusions

Addition of ALP to chitosan/β-GP solutions not only induces hydrogel mineralization, but also accelerates gelation upon heating to body temperature. Extent of acceleration is dependent on the chitosan preparation used.

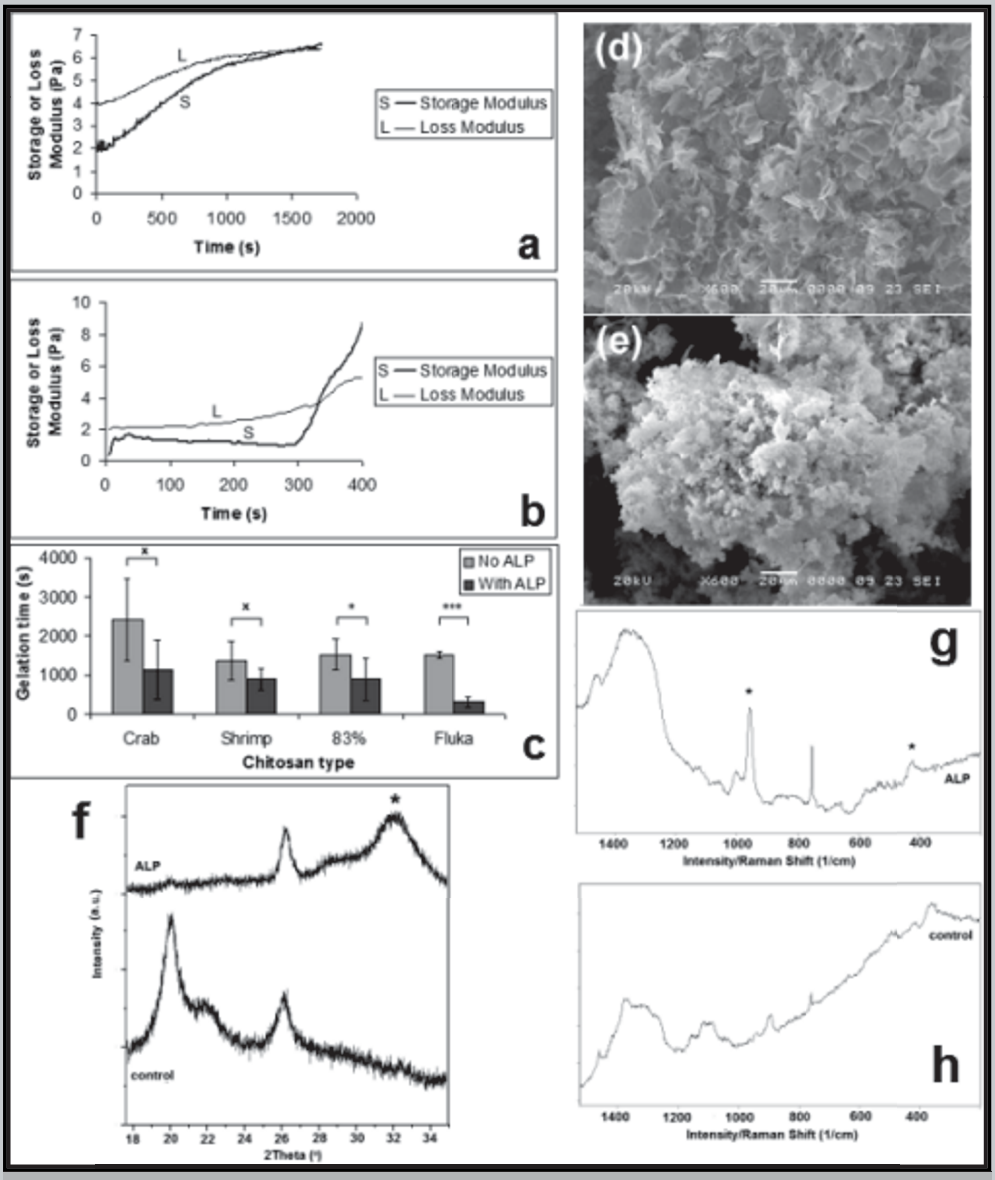

FIGURE (a,b,c) Gel transition point (crossover of G' (storage modulus) and G'' (loss modulus)) was reached more quickly after addition of ALP. SEM revealed mineral deposits on gels containing ALP (e) which were absent in gels without ALP (d). XRD (f) and Raman (g,h) revealed peaks characteristic for apatite (*) in gels containing ALP which were absent in gels without ALP.

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Piśmiennictwo

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