

Acceleration of gelation and mineralization of chitosan hydrogels by alkaline phosphatase

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Introduction Thermosensitive chitosan hydrogels which gel in the range 30-60 °C can be formed by neutralization of an acidic chitosan solution by addition of sodium beta-glycerophosphate (Na-GP). In this study alkaline phosphatase (ALP) was incorporated into such hydrogels to promote their mineralization for bone regeneration applications. **Materials and Methods** Chitosan, Na-GP and ALP solutions were mixed to yield gels with concentrations 20.5 mg/ml, 90.9 mg/ml and 0 or 0.23 mg/ml, respectively. After overnight gelation at 37 °C, mineralization was induced by incubation in 0.1 M calcium glycerophosphate (Ca-GP) for 10 d followed by rinsing and incubation in Milli-Q water for 24 h.. Rheological time sweeps took place at 37 °C at an oscillatory stress of 5 Pa and frequency of 1 Hz. The gelation point was defined as the point where the storage modulus (G') exceeded the value of the loss modulus (G'') (Figure a,b). Formation of CaP was demonstrated by FTIR, Raman, XRD, SEM and ICP-OES. **Results and Discussion** ALP accelerated gelation of four chitosan preparations (Figure a,b,c) which demonstrates the feasibility of ALP-accelerated gelation for different preparations. Mineralization of chitosan hydrogels by ALP was demonstrated visually by SEM (Figure d,e) and 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. **Conclusion.** Addition of ALP to chitosan/ β -GP solutions induces hydrogel mineralization and accelerates gelation.

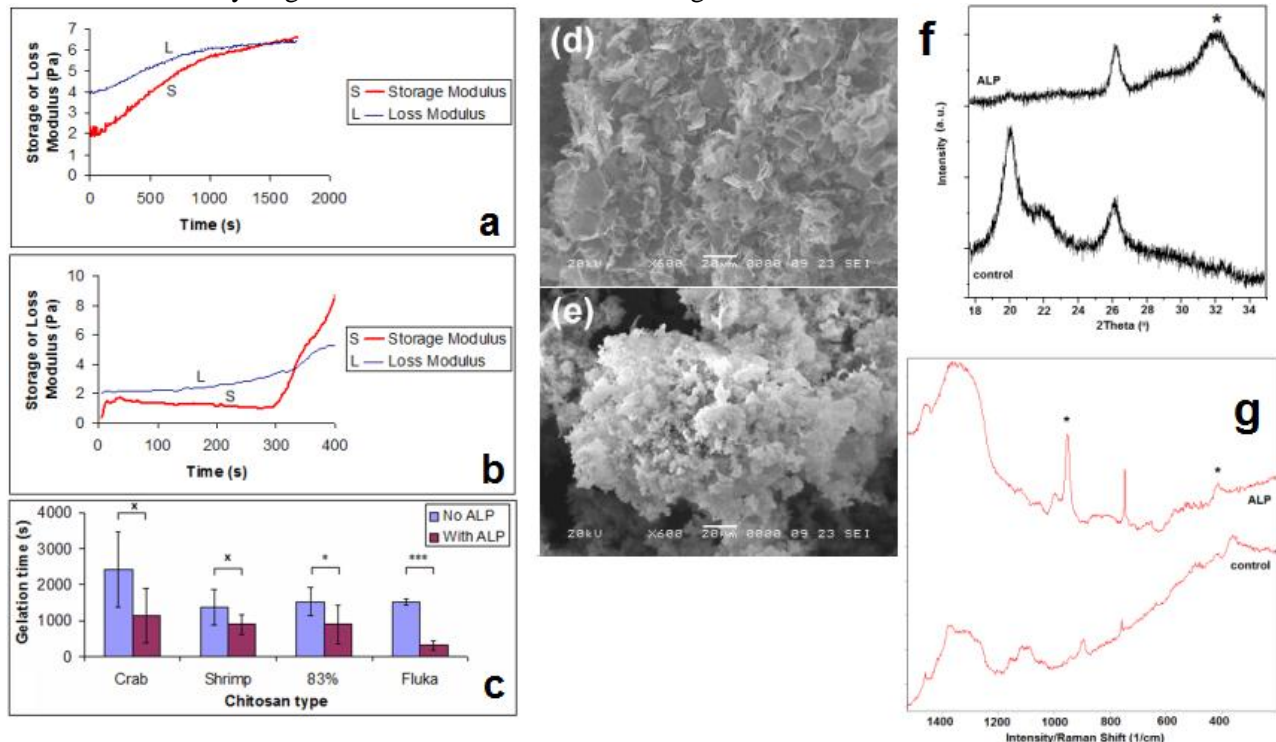


Figure. (a,b,c) Gelation time (crossover of G' and G''). SEM of gels without (d) and with ALP (e). XRD (f) and Raman (g,h) spectra with peaks characteristic for apatite (*)