Injectable thermosensitive chitosan hydrogels with enhanced properties

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Statement of Purpose: Injectable hydrogels such as chitosan-β-glycerol phosphate (CH-BGP) are increasingly used in biomedical applications since they provide an excellent platform for a less invasive treatment and/or a more local delivery of cells, drugs and/or other bioactive products. However, there is a lack of injectable material that combines rapid gelation, high mechanical strength, and excellent cell-compatibility. We here reported a new combination of gelling agent which enables to accelerate gelation and drastically increase mechanical resistance while reducing the total salt concentration and related cytotoxicity as evaluated on L929 mouse fibroblasts in vitro.

Note: Hydrogels are named according to the gelling agent and its concentration. e.g. CH:SHC005:PB004pH8 represents a hydrogel containing chitosan, 0.05 M SHC and 0.04 M PB at pH 8.

Methods: To prepare a hydrogel (2% w/v), an acidic solution of chitosan was mixed with a solution containing one or two gelling agents chosen among BGP, sodium hydrogen carbonate (SHC) and phosphate buffer (PB). The rheological properties of the hydrogels were studied at room and body temperature and during temperature ramps using a rheometer equipped with a co-axial cylinder geometry while axial unconfined compression tests (up to 50% deformation) were performed using Bose ElectroForce® 3200 instrument. The FTIR spectra were obtained using a Nicolet 6700 FTIR spectrometer and KBr pellets. The cytotoxicity of hydrogel extracts was evaluated in vitro using L929 cells.

Results: Upon heating from 5 to 65 °C, the temperature at which G' rapidly increased and the slope of this increase were influenced by the kind and concentration of gelling agents (Fig. 1). G' increased much more rapidly and extensively in gels prepared with SHC combined or not with PB and BGP, compared to BGP or PB alone or their combination. In gels prepared with SHC0075, G' increased strongly to reach about 14 kPa, but only at a temperature > 40 °C. With SHC005:PB004pH8 and SHC005:BGP01, G' started to increase remarkably at temperature > 25 °C. In contrast, with PB and BGP alone, G' values were still below 2.5 kPa at the end of the test. With PB008pH8, G' increased slowly at 15 °C, while with BGP04 it increased at 25 °C. Excepted for CH:SHC, G' profiles showed more than one stage, which may be due to the presence of more than one basicity in the medium.

All the samples with combination containing SHC (SHC:PB or SHC:BGP) showed remarkably higher secant moduli than those obtained with BGP04, PB008pH8 or BGP02:PB004pH8 (Fig. 2) and they kept their initial cylindrical shape after compression, while the latter hydrogels broke up during compression. Using SHC alone at a specific concentration led to the formation of a resistant hydrogel, but only after several hours at 37 °C. The addition of SHC to other gelling agents is a key factor to simultaneously control the gelation rate and increase the strength of the hydrogel. Hydrogels obtained in presence of SHC showed porous structures and no cytotoxicity on L929 cells was noticed, in contrast to BGP gels which led to significant cell death at concentration above 0.4 M. FTIR indicated that salts were removed from hydrogel by washing. These properties are favorable to drug release, cell invasion and tissue regeneration.

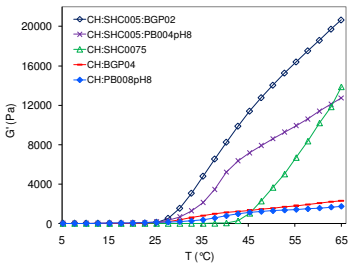

Figure 1. Temperature dependence of storage and loss moduli (G', G") of CH hydrogels upon heating from 5 to 65 °C at the rate of 1 °C/min. (n = 3, mean values).

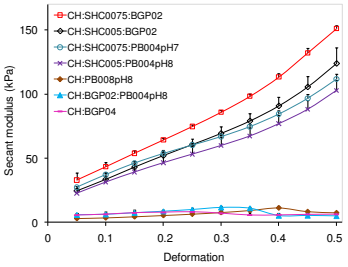

Figure 2. Variation of secant Young's modulus in compression at 5-50% deformation (100% deformation/min) for various CH hydrogels incubated at 37 °C for 24 h. (n = 3, mean ± SD).

Conclusions: The strength of chitosan hydrogels was remarkably increased without modifying chitosan, using ionic or covalent crosslinking or increasing the ions concentration. A very interesting synergic effect was observed when combining SHC with BGP or PB, despite even lowered salt concentration. The hydrogels presented physiological pH and porous structure and they were no cytotoxic. The properties of the obtained injectable hydrogels are interesting for various pharmaceutical and biomedical applications, such as drug delivery and embolization of blood vessels. The idea of mixing SHC with BGP or PB to optimize or improve the gelation rate and the strength of hydrogel may be generalized to other weak bases or even to other polymers.

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