PEG-grafted-chitosan hydrogel suitable for non-viral gene delivery

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Statement of Purpose: Injection of *in situ* gel-forming biopolymers is becoming increasingly attractive for the development of *in situ* gene and drug delivery systems. Moreover, thermosensitive polymer hydrogels that undergo a sol-gel transition in response to temperature changes are of great interest in therapeutic delivery and tissue engineering as injectable depot systems. In the present study a chitosan-based, injectable thermogel was prepared by grafting an appropriate amount of polyethyleneglycol (PEG) onto the chitosan backbone and studied for lipoplex (lipid/DNA complex) release *in vitro*.

Methods: PEG grafted chitosan (PEG-g-chitosan) was synthesized according to Bhattarai et al.[1] Briefly PEG-aldehyde was prepared by oxidation of PEG (MW 2000) with DMSO/acetic anhydride. PEG-grafted chitosan (PEG-g-chitosan) was prepared by alkylation of chitosan (medium MW, 75-85% deacetylated) followed by Schiff base formation. Cyanoborohydride ($NaCNBH_3$) solution was then added into the mixture of PEG-aldehyde and chitosan (molar ratio:0.4/1) and left stirring for 4 days. The resultant mixture was dialyzed against distilled water for 24 h and 0.05 M NaOH for 8 h, and the solution was freeze-dried. PEG-grafted chitosan was obtained by removal of unreacted PEG from the freeze-dried samples with excess acetone. ^{1}H-NMR and infrared spectroscopy (I.R.) were used to characterize PEG-g-chitosan.

Lipoplex solution was prepared at r.t. in deionized H_2O by addition of plasmid DNA (pEGFP) to the cationic lipid solution at the desired lipid concentration to obtain the charge ratio (CR, +/-) of 10.[2]

Hydrogels were obtained dissolving PEG-g-chitosan either in deionized H_2O or in lipoplex solution (DNA 6.67 ng/µl) at different polymer weight percentages (1.5% and 3% w/v) and centrifuged at 4°C to remove bubbles. Gelation behaviour of PEG-g-chitosan alone or with lipoplexes was studied at a temperature range of 10°C–45°C. Cytotoxicity was evaluated in L929 cell line with Alamar blue viability assay. Extracts for indirect tests were obtained from material under standardized conditions (ISO 10993-5). Degradation tests were carried out over 30 days in PBS at 37°C.

Results: A comparative I.R. spectrum study of PEG-g-chitosan, chitosan, and PEG, confirmed the success of grafting PEG to chitosan. The absorption bands of 1280 cm^{-1}, 947 cm^{-1}, 842 cm^{-1}, characteristics of pure PEG, were found in PEG-g-chitosan spectrum. The degree of PEG substitution was evaluated through the integration of the anomeric protons of PEG-g-chitosan (5.3 pp, 0.2H) and unPEGylated chitosan (5.3-5.2 ppm, 1H), corresponding to a molar ratio of 0.17.

There was a minimum concentration of PEG-g-chitosan under which there was no sol-gel transition although the temperature was risen. Indeed at 1.5% w/v of copolymer in deionized H_2O no apparent gelation was observed. On the other hand, when the temperature was increased from 10°C to 40°C, PEG-g-chitosan 3% w/v in deionized H_2O underwent to sol-gel transition, as shown in Figure 1. Below the transition temperature, the solution was a viscous liquid that flowed easily and was injectable through a 20-gauge needle. As the solution was heated to body temperature, it transformed into gel. This behavior was observed by tilting or inverting the test tube containing the hydrogel at different temperatures.

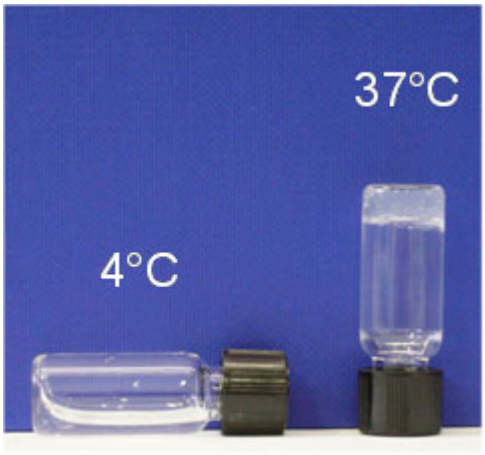

Figure 1. Photo of 3% w/v PEG-g-chitosan in deionized H_2O before (4°C) and after (37°C) gelation.

The *in vitro* indirect cytotoxicity test was performed using the mouse fibroblasts L929 cell line. The absorbance values obtained for PEG-g-chitosan extracts resulted comparable to the negative control (medium without material extracts) for all the three time points tested (1, 2 and 3 days). The extracts induced neither cell viability reduction nor inhibition of cell growth resulting to have no toxic effects. Moreover, the hydrogel displayed no apparent degradation in PBS after 30 days.

The presence of lipoplexes in 3% w/v copolymer solution did not influence the temperature gelation and the stability behavior of the PEG-g-chitosan hydrogel.

Conclusions: We synthesized PEG-g-chitosan copolymer capable of thermosensitive transition from a solution at low temperature to an opalescent gel at approximately 37°C, and thus it gelled readily at body temperature. This, combined with biocompatible and biodegradable nature of both chitosan and PEG, made the hydrogel particularly suitable for *in vivo* medical applications such as sustained localized gene release.

Transfection activity of 3% w/v PEG-g-chitosan in lipoplex solution and the kinetic of transgene release are currently under investigation.

References:

[1] Bhattarai N. J Control Release. 2005; 103(3): 609-24.
[2] Candiani G. J Gene Med. 2008; 10(6): 637-45.