Injectable Thermosensitive Poly(Ethylene Glycol)/Polypeptide Block Copolymer Hydrogels for Localized Anti-Cancer Drug Delivery

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ABSTRACT SUMMARY

Injectable thermo-sensitive hydrogels based on PEG/poly(γ-ethyl-L-glutamate) triblock copolymers were developed. The PEG/polypeptide hydrogels displayed good biocompatibility in vitro and in vivo. The degradation of the hydrogels was accelerated by addition of enzymes in vitro and the hydrogels degraded gradually after being subcutaneously injected into rats. Paclitaxel (PTX)-loaded PEG/polypeptide hydrogels exhibited significantly higher and longer tumor suppression efficacy compared to a commercially available formulation of PTX solution.

INTRODUCTION

Thermo-responsive injectable hydrogels have received extensive investigation in the past decade, due to their potential applications in localized drug delivery and regenerative medicine [1-3]. Typical biodegradable thermo-gelling copolymers are composed of a hydrophilic poly(ethylene glycol) (PEG) block and a hydrophobic biodegradable block, such as poly (lactic acid) (PLA), poly(lactic acid-co-glycolic acid) (PLGA), poly(ε-caprolactone) (PCL), poly(ε-caprolactone-co-lactide) (PCLA), poly((R)-3-hydroxybutyrate) (PHB), poly(amidoamine) (PAA), etc. Very recently, thermo-gelling amphiphilic block copolymers comprising PEG and a hydrophobic polypeptide block, such as poly(L-alanine), poly(L-alanine-co-L-phenylalanine) and poly(γ-alkyl-L-glutamate) (PALG), have attracted considerable interest attributed to their markedly lower gelation concentration compared to PEG/polyester systems and a unique secondary conformation change during the gelation [4,5]. In addition, polypeptide-based hydrogels cause no acidic microenvironment after degradation, which may be beneficial to maintaining the activity of bioactive molecules and cells encapsulated within the hydrogels as well as to reducing the inflammatory reaction of surrounding tissues. In our recent work, a thermo-gelling PEG/poly(γ-alkyl-L-glutamate) (PALG) diblock copolymer was developed, and an interesting effect of hydrophobic side chain on the hydrogelation of PEG-polypeptide diblock copolymers was revealed [5]. In the present work, thermo-sensitive poly(γ-ethyl-L-glutamate)-b-PEG-b-poly(γ-ethyl-L-glutamate) (PELG-PEG-PELG) triblock copolymer hydrogels were developed. The biocompatibility and biodegradation of the polypeptide hydrogels were evaluated in vitro and in vivo, and the localized anti-tumor efficacy of paclitaxel (PTX)-loaded polypeptide hydrogels was investigated.

EXPERIMENTAL METHODS

PELG-PEG-PELG triblock copolymers were synthesized via ring opening polymerization (ROP) of γ-ethyl-L-glutamate N-carboxy anhydride (NCA) using amino-terminated PEG as a macro-initiator (Scheme 1) [5]. The resulting copolymers were characterized by 1H NMR and GPC. The critical micelle concentrations (CMC) of the copolymers were investigated by the pyrene-probe-based fluorescence technique. The sol-gel transition of the polymer aqueous solution was determined by a tube-invert method and rheological measurement.

In vitro cytotoxicity of the copolymers was evaluated by MTT assay. For in vivo biodegradation and biocompatibility tests, the polypeptide hydrogels were subcutaneously injected into SD rats. The integrity of the hydrogels was checked every week in vivo and the response of surrounding tissues to the materials was measured by histological analysis. The localized anti-tumor efficacy was investigated after injection of paclitaxel (PTX)-loaded polypeptide hydrogels near the tumors in female BALB/c nude mice xenoimplanted with HepG2 cells.

Scheme 1. Synthetic route of PELG-PEG-PELG triblock copolymer.

RESULTS AND DISCUSSION

PELG-PEG-PELG triblock copolymers were synthesized via ring opening polymerization (ROP) of γ-ethyl-L-glutamate N-carboxy anhydride (NCA) using amino-terminated PEG as a macro-initiator. The polydispersities (PDIs) of all the resulting products determined by GPC were in the range of 1.2 – 1.3, indicating that the triblock copolymers were all well prepared. The composition and block length of the copolymer could be tailored by varying the feed ratio. The aqueous solutions of the PELG-PEG-PELG triblock copolymers exhibited thermo-induced sol-to-gel transitions at relatively lower polymer concentrations (≥2%). The sol-gel transition temperature and critical gelation concentration (CGC) were obviously influenced by relative block length. The gelation temperature can be controlled in the range of 10 – 30 °C by changing the
composition of the copolymer as well as the polymer concentration.

In vitro degradation of the PEG/polypeptide hydrogels was found to be markedly accelerated by addition of proteinase K. After subcutaneously injection of the polymer solutions into SD rats, hydrogels were formed in situ in a short time. A 4.0 wt% polypeptide hydrogel was found to degrade completely within 3 weeks in rats. Histological analysis indicated that even though mild acute inflammatory response was observed at 1 week post-injection, the inflammatory response was reduced and eliminated gradually along with the degradation of the hydrogels and no chronic inflammation was observed. This indicated that the PEG/polypeptide hydrogels were acceptable in vivo.

After injection of paclitaxel (PTX)-loaded polypeptide hydrogels near the tumors in female BALB/c nude mice xenoimplanted with HepG2 cells, the PTX-loaded hydrogels treated group showed markedly higher and longer tumor suppression efficiency compared to the group treated with a commercially available formulation of PTX solution (Taxol). The enhanced anti-tumor efficacy of the PTX-loaded hydrogels is likely attributed to a more sustained and longer PTX release profile. Additionally, analysis of several clinical chemical parameters that are associated with the functions of some major organs indicated that the PTX-loaded hydrogels displayed no serious damage to normal organs during the treatments.

CONCLUSION
The PEG/polypeptide triblock copolymers can form hydrogels at much lower polymer concentrations, compared to PEG/polyester systems. The polypeptide hydrogels indicated good biocompatibility in vitro and in vivo, and the in vivo degradation of a 4.0 wt% PEG/polypeptide hydrogel was found to last 3 weeks. After injection of PTX-loaded polypeptide hydrogels near the tumors in tumor-bearing mice, significantly higher and longer tumor suppression efficiency was achieved compared to the group treated by using a commercially available PTX formulation. Therefore, the presented injectable polypeptide hydrogels can serve as a promising platform for localized anti-cancer drug delivery.

REFERENCES

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