Intravitreal injectable hydrogel incorporating microgel for prolonged protein delivery

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Statement of Purpose: Age-related macular degeneration (AMD) is a major reason of visual impairment including vision loss, blurred and finally irreversible blindness. Currently, intravitreal injection of anti-VEGF is the primary therapy for treatment of AMD. However, the injection is needed by monthly for a satisfactory treatment, which significantly enhances the risk of complications, patients' psychological and economic burdens.[1] Moreover, frequent intravitreal injection for effective dose can cause not only serious side effects such as endophthalmitis and increased intraocular pressure but also systemic adverse phenomenon such as myocardial infarction, stroke and kidney disease.[2] To overcome these, we report an injectable hydrogel incorporating protein-loaded microgel that can serve as an intravitreal controlled and long-term protein carrier which can be administrated for the treatment of neovascular retinal diseases such as AMD (Fig. 1).

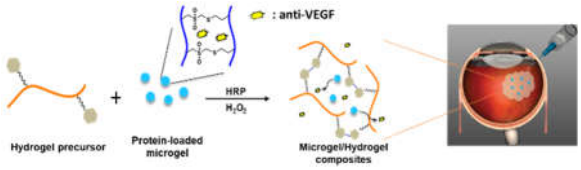
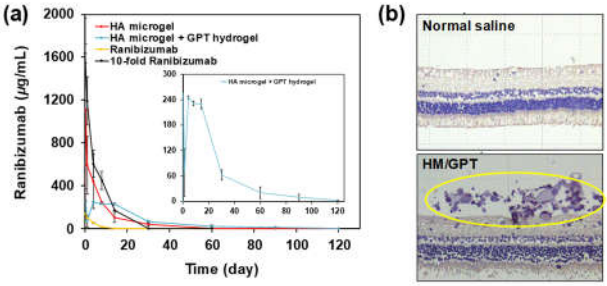


Figure 1. Schematic representation of intravitreal anti-VEGF releasing microgel/hydrogel composites in a sustained manner.

Methods: HA was modified with divinyl sulfone and cystamine dihydrochloride by click chemistry and EDC/NHS-mediated reaction, termed as HA-VS and HA-SH, respectively. GPT conjugates were synthesized and characterized as previously reported.[3] The HA microgel was prepared by emulsification *via* thiol-ene click reaction. The characteristics of microgel were optimized by varying concentration and size of microgels. *In situ* forming GPT hydrogel incorporating HA microgel (HA/GPT composite gel) was fabricated by enzyme-mediated crosslinking of HRP/H_2O_2. The *in vitro* prolonged ranibizumab (RBZ) release rate of HA/GPT composite gel was evaluated compared to controls by ELISA. The *in vivo* pharmacokinetic (PK) was evaluated followed by intravitreal injection in non-diseased New Zealand White (NZW) rabbit model. To assess *in vivo* PK, RBZ concentration of vitreous, retina, anterior and plasma were investigated by ELISA followed by intravitreal injection in non-diseased New Zealand White (NZW) rabbit model. The histological analysis was performed by TUNEL assay.

Results: The successful synthesis of HA-VS and HA-SH was confirmed by ^{1}H NMR and Ellman's assay. The loading (52 - 99%) and release rate (41 - 86% for 30 days) of BCZ could be controlled by adjusting size and concentration of HA microgels. The *in vitro* released BCZ showed efficacy by inactivating VEGF for 30 days by ELISA and inhibiting HUVECs growth. The physico-chemical properties of HA/GPT composite gel were controllable by varying concentrations of HRP and H_2O_2. The *in vitro* RBZ release could be sustained up to 90 days from the HA/GPT composite gel, significantly prolonged than that of only microgel or hydrogel. *In vivo* PK was evaluated that the initial burst release was minimized and the therapeutic concentration of RBZ in vitreous were remained for 120 days from the HA/GPT composite gel compared to controls (Fig. 2a). Moreover, histological analysis showed non-apoptosis and aggregates nearby retinal layer (yellow circle), facilitating accumulated protein to the retina (Fig. 2b).

Figure 2. (a) *In vivo* PK in vitreous humor after intravitreal injection



for 120 days (b) Histological analysis of retinal layer at 60 days after intravitreal injection.

Conclusions: A novel polymeric microgel/hydrogel composite was developed for prolonged intravitreal protein delivery. The HM/GPT significantly suppressed initial burst and extended protein delivery in eyes to minimize the adverse effects. In addition, the accumulation of Rb in retina up to 120 days could be explained due to the aggregates of HM/GPT nearby retina. Therefore, we expect that HM/GPT as a promising carrier to overcome the current challenges of intravitreal anti-VEGF therapy for the treatment of AMD.

References: [1] Y. Yu TVST 2015;4(2);5. [2] K.G. Falavarjani Eye 2013;27(7);787-794. [3] Y. Lee J Mater Chem B. 2013;1;2407-24

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