**Sustained Release of Bevacizumab Using an Injectable, Degradable and Biocompatible Reverse Thermal Gel**

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**Statement of Purpose:** Treatment of uncontrolled neovascularization in ocular conditions is limited by the rapid clearance of anti-angiogenic therapies such as bevacizumab (Avastin). In humans the half life of bevacizumab is on the order of 7 days, necessitating frequent, painful injections.[1] As such, sustained release systems for intraocular applications are highly desirable for they can minimize patient burden while improving therapeutic efficacy. Ideally, such systems should be injectable, biocompatible, biodegradable and able to protect drug activity for sustained release. The objective of this study is to investigate the ability of a reverse thermal gel, poly(ethylene glycol)-poly(serinol hexamethylene urethane) (ESHU), to sustain the release of bevacizumab in vitro and in vivo.

**Methods:** 10, 15 or 20% solutions of ESHU containing 25, 50 or 100 mg/ml of bevacizumab were prepared for in vitro release studies. 50 µl doses were injected into a 1% solution of hyaluronic acid (HA) to mimic vitreous humor composition[2], and samples were kept at 37 °C under gentle agitation to simulate eye motion. Supernatant samples were obtained at 1, 3 and 7d and weekly thereafter. An enzyme-linked immunosorbent assay (ELISA) was used to determine the concentration of bevacizumab. For in vivo studies, a 50 µl dose of ESHU containing 1.25 mg bevacizumab or 1.25 mg of free bevacizumab was injected intravitreally into rabbit eyes (n=4 eyes per group). This amount was chosen as it is the standard dose administered by ophthalmologists in humans.[3] We monitored intraocular pressure, observed overall fundus health by indirect ophthalmoscopy and sampled anterior chamber fluid by paracentesis at 1, 3 and 7 days, and weekly for 12 weeks. ELISA was used to determine the amount of bevacizumab in samples. After sacrifice, histological analysis was performed to assess tissue morphology and to identify potential inflammation.

**Results:** ESHU is capable of sustaining bevacizumab release over approximately three months in vitro and exhibits dose- and concentration-dependent release behavior. In vivo, ESHU formulations are easily injected through at 31G needle, and form a physical gel almost immediately within the vitreous cavity. Indirect observation revealed minimal evidence of inflammation throughout the study period, and intraocular pressure measurements remained at baseline levels. The retina remained intact and the vitreous was cell-free, indicating that ESHU did not elicit a significant inflammatory response (**Figure 1**). Preliminary release data suggests that free bevacizumab is completely cleared and undetectable by ELISA at one month, while ESHU-delivered bevacizumab remains present in the eye for 8 weeks.

**Conclusions:** Taken together, the results suggest that ESHU is a promising drug delivery platform for intravitreal applications as it is minimally invasive, biocompatible and allows for sustained release of bevacizumab both in vitro and in vivo.

**References:**

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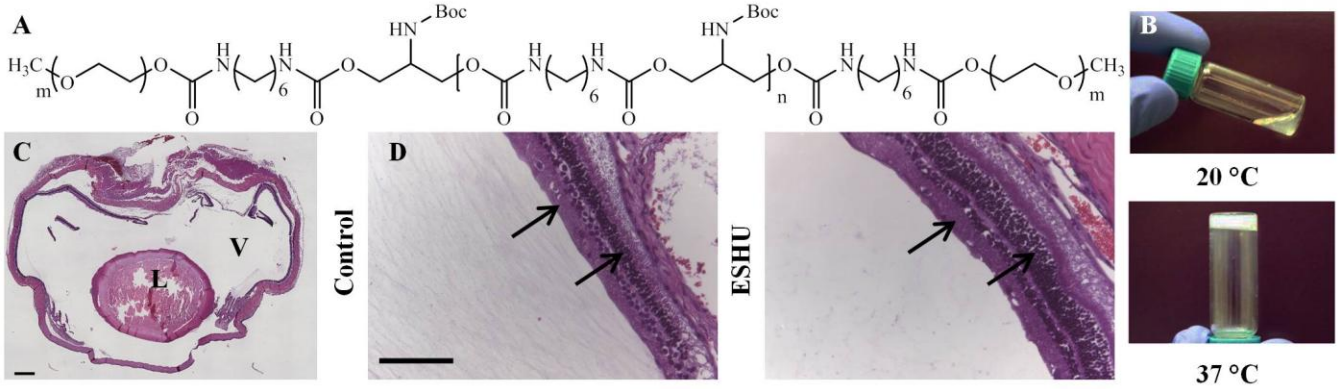


**Figure 1. (A)** ESHU is an ABA-type block copolymer composed of hydrophilic poly(ethylene glycol) end groups and a hydrophobic polyurethane middle block. **(B)** At room temperature, a 15% solution of ESHU remains in a liquid state. When placed at 37°C, a physical hydrogel forms rapidly. **(C)** Intravitreal injection does not affect the overall morphology of the eye as demonstrated by H&E staining. L: lens, V: intravitreal space. Scale bar 1 mm. **(D)** ESHU causes minimal inflammatory response, as the layers of the retina (arrows) are intact and distinct, while the vitreous humor remained cell-free. There is no difference between ESHU (right) and control (left) groups. Scale bar 100 µm.