Supporting Information

Engineering an Injectable and Tunable Hydrogel as a Potential Vitreous Substitute

Ting Wang[A], Lang Qin[B], Yuanyuan Ding[B], Jing Li[B], Hanyue Xu[A], Bin He[B], Jun Cao[B,*], Ming Zhang[A,*]

[A]Department of Ophthalmology, West China Hospital, Sichuan University, No. 37 Guoxue Alley, Wuhou District, Chengdu, 610041, Sichuan, China.

[B]National Engineering Research Center for Biomaterials, Sichuan University, Chengdu 610041, Chengdu, 610041, Sichuan, China.

* Corresponding author

Method

Quantitative Assessment of Functional Group Conversion

The concentration of residual thiol groups was determined using the Ellman's assay. A series of L-cysteine standard solutions with concentrations ranging from 0.05 to 2.0 mM was prepared in PBS buffer to establish a standard calibration curve. For sample analysis, a specified amount of the diluted hydrogel solution was mixed with DTNB solution (2 mg/mL) in PBS buffer. After incubation at room temperature for 15 minutes, the absorbance of the mixture was measured at 412 nm using a UV-Vis spectrophotometer. The concentration of residual thiol groups in the samples was then calculated according to the established linear regression equation.

The residual concentrations of maleimide (MAL) groups were quantified using UV-Vis spectrophotometry (Evolution Pro, Thermo Scientific, USA). 8sPEG-MAL exhibits a characteristic absorption peak at approximately 300 nm, which shifts and is typically monitored at 310 nm to avoid background interference. This absorbance decreases as MAL groups are consumed during the crosslinking reaction with thiol group. To determine MAL concentrations, a standard curve was established at 310 nm. Samples were measured in a quartz cuvette, and the resulting absorbance values were used for quantification based on the regression equation.

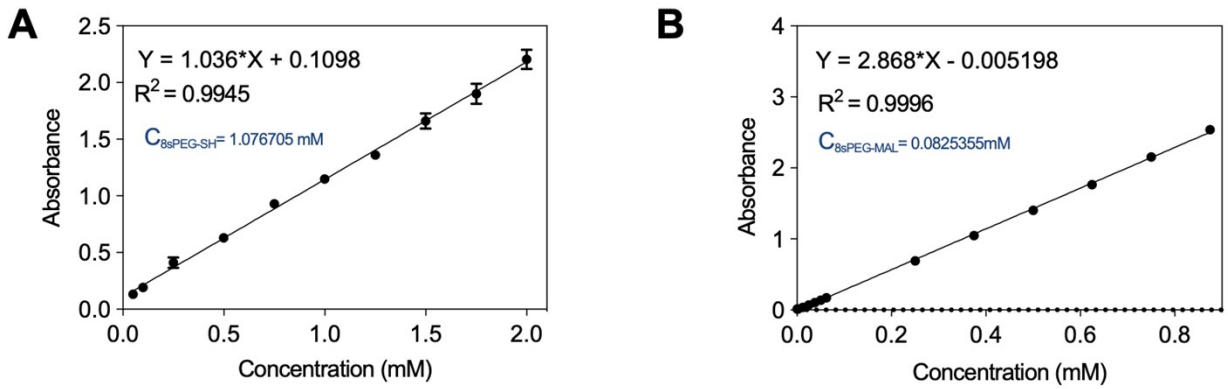

Figure S1. Quantitative determination of functional group concentrations for 8s PEG derivatives. (A) Standard curve of L-cysteine measured at 412 nm using a microplate reader (Ellman's assay). Data are presented as Mean ± SD (n=4). (B) Standard curve of 8sPEG-MAL determined by UV-Vis spectrophotometry at 310 nm. The blue value represents the measured concentration of residual maleimide (-MAL) groups.

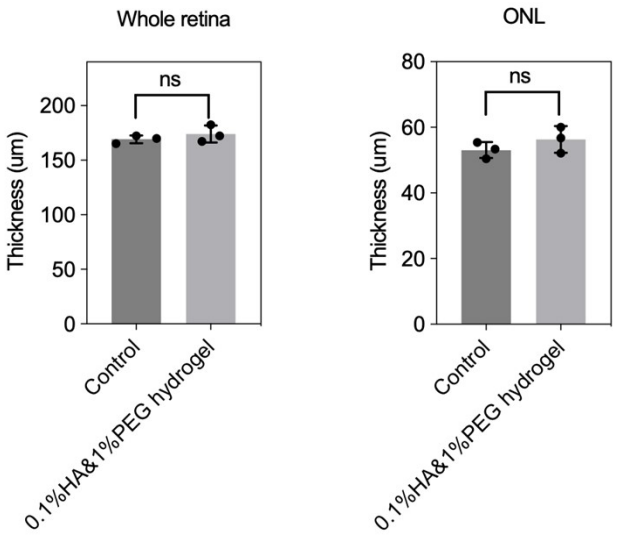

Figure S2. Quantitative morphometric analysis of retinal structure. The thicknesses of the whole retina and the outer nuclear layer (ONL) were measured 14 days post-injection to evaluate the structural integrity. Data are presented as mean ± SD (n = 3). ns indicates no significant difference ($p > 0.05$) determined by Student's t-test.

Table S1. Clinical Ocular Safety Scores (McDonald-Shadduck) of the Control and Gel Groups at Different Time Points.

Time Point		Cornea	Iris	Flare	Conjunctiva
Day 1	Control	0.3 ± 0.1	0.2 ± 0.4	0.1 ± 0.3	0.5 ± 0.3
	Gel	0.3 ± 0.1	0.4 ± 0.2	0.2 ± 0.1	0.4 ± 0.2
Day 3	Control	0 ± 0	0 ± 0	0 ± 0	0.2 ± 0.1
	Gel	0 ± 0	0 ± 0	0 ± 0	0.2 ± 0.2
Day 7	Control	0 ± 0	0 ± 0	0 ± 0	0 ± 0
	Gel	0 ± 0	0 ± 0	0 ± 0	0 ± 0
Day 14	Control	0 ± 0	0 ± 0	0 ± 0	0 ± 0
	Gel	0 ± 0	0 ± 0	0 ± 0	0 ± 0