Electronic Supplementary Information (ESI)

Super-fast in-situ forming hydrogel based on multi-arm functional polyethylene glycol as an endotamponade substitute

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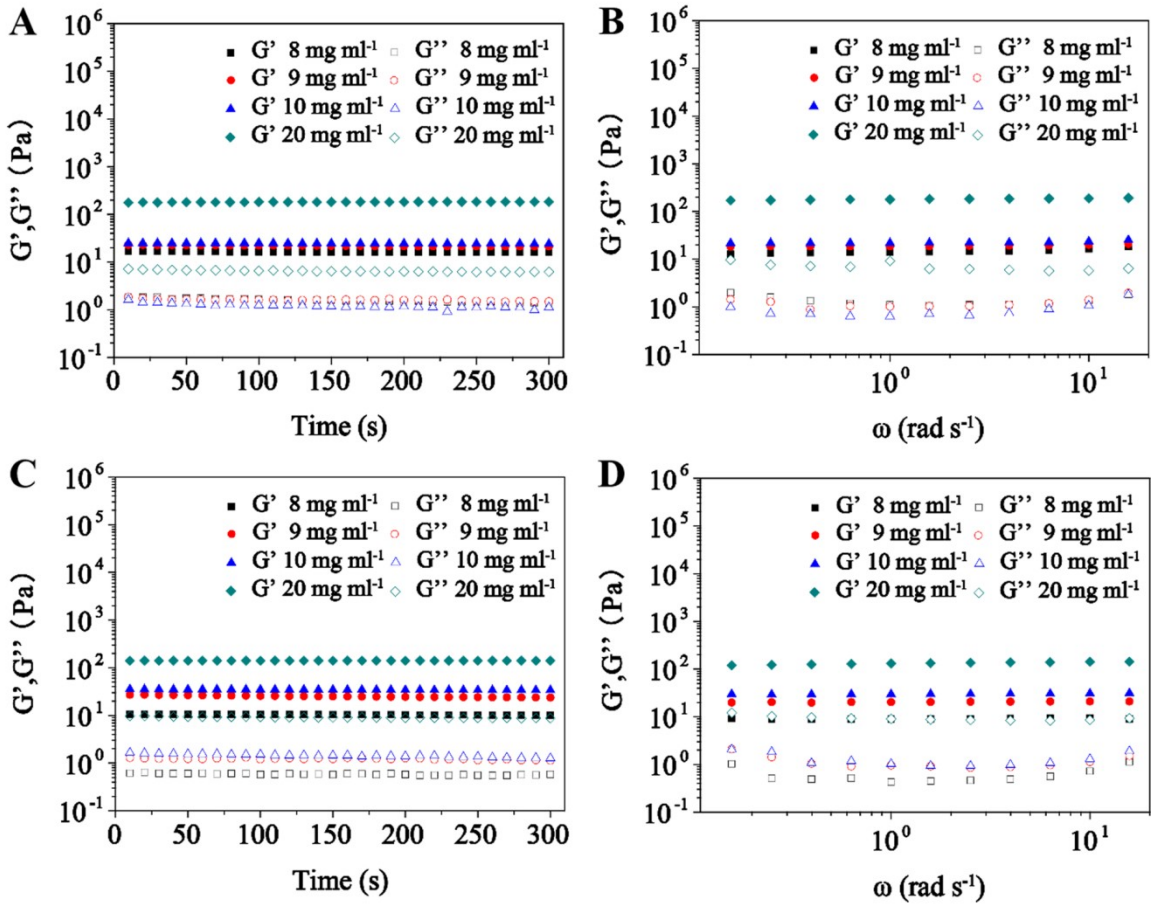

Fig. S1. Rheological properties (G' and G'') were measured again，when the various concentrations of hydrogels were placed in vitro at A, B) one month and C, D) six months.

Cytotoxicity study in vitro

The cytotoxicity of hydrogels was assayed by testing the extracts of the hydrogels with Rhesus macaque choroid-retinal endothelial cells (RF/6A) and Adult Retinal Pigment Epithelial cell lines-19 (ARPE-19) (Jennio Biological Technology, Co., Ltd, Guangzhou, China) with a colorimetric cell-counting kit (CCK-8). These cells were identified without mycoplasma contamination. When the experiment started, the cells were seeded in T-75 vial after resuscitation, cultured in a CO_2 incubator (37℃±2 ℃, 5%±2% CO_2) for passages. The passages cells of RF/6A and ARPE-19 were cultured in complete DMEM and RPMI1640 medium, respectively, and then used for formal experiments. Each cell line was co-cultured with the BSS group (control) and the extracts of AVB groups. 100μl cell suspension was seeded in 96-well culture plates when the density reached the level of 1×10^5 cells ml^{-1}. The extracts were obtained by incubating the hydrogel in the culture medium containing 10% fetal bovine serum (FBS), the extraction ratio was 3 cm^2 ml^{-1} for 48 hours (±30 mins) at 37 ℃，according to the ISO standards (GB/T 16886.5-2017/ISO 10993-5:2009).[1] When the cells were approximately 50% confluent, added 100 μl drug inducers (extracts or BSS) into the culture dish after removing the culture medium, and then incubated with the cells together for 48 hours (±30 mins). According to the instructions, the cell viability was assayed with a CCK-8 (Beyotime Biotechnology, In China). The cell morphology was analyzed under an inverted microscope.

Characteristics of the hydrogels

The physical properties of the hydrogel are shown in Table S1, including refractive index, pH, and water content. The results proved that our hydrogels resembled the natural vitreous body both in physical features and parameters.

Table S1. Refractive index, pH and water content of hydrogels (the data represent the mean ± SD，n=6).

Samples	Refractive index	PH	Water content
8 mg ml^{-1}	1.3364±0.0002	6.87±0.04	98.00±0.41%
9 mg ml^{-1}	1.3365±0.0001	6.86±0.03	97.29±0.26%
10 mg ml^{-1}	1.3366±0.0001	6.81±0.04	97.49±0.45%
20 mg ml^{-1}	1.3390±0.004	6.70±0.08	97.33±0.48%
Human vitreous[2,3]	1.3345-1.3348	7.0–7.4	98-99%

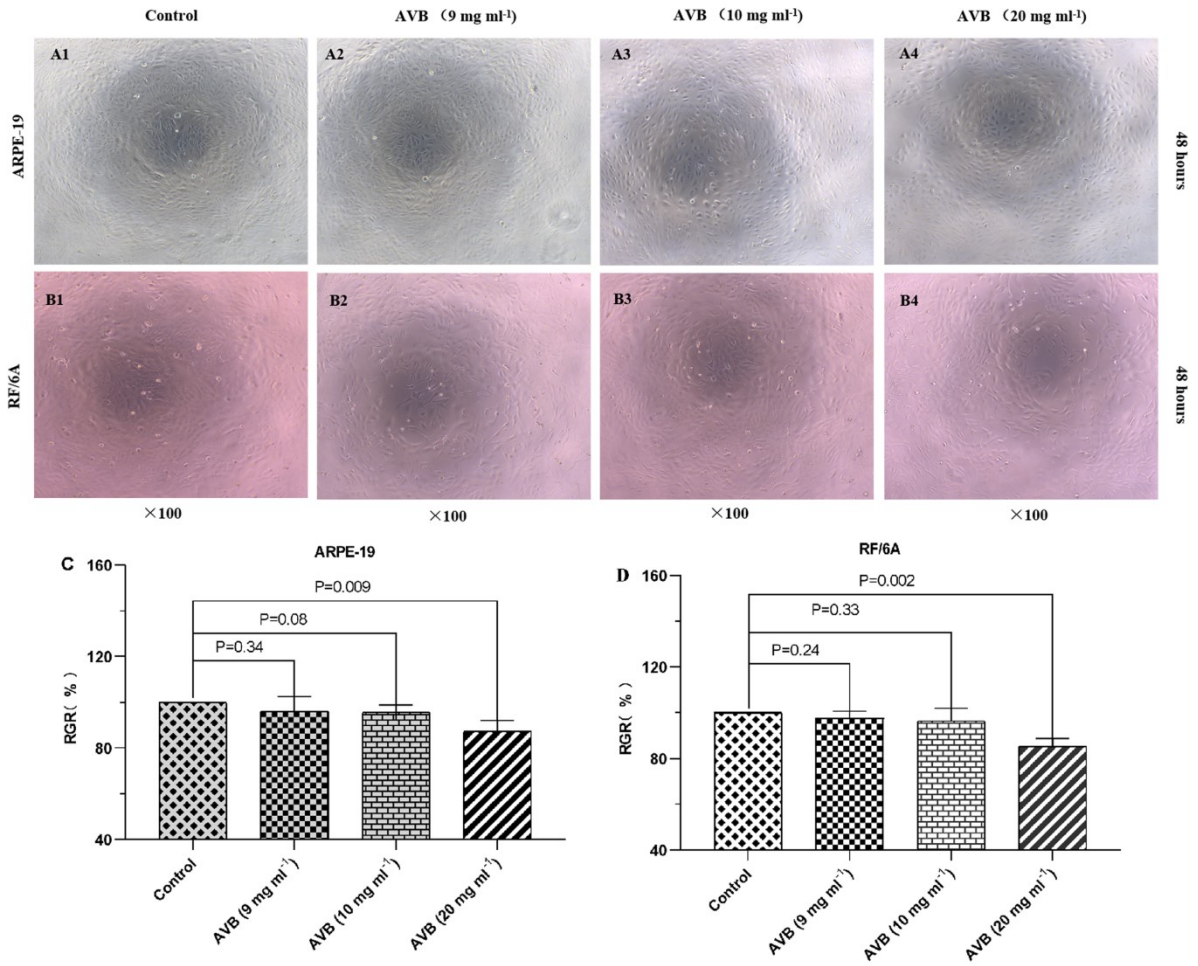

Fig. S2. The morphology of A1-A4) ARPE-19 and B1-B4) RF/6A cells cultured with BSS (control), extracts of AVB for 48 hours. The RGR of C) ARPE-19 and D) RF/6A cells were analyzed between extracts of AVB groups and the control group.

The major reason for the failure of the implantable vitreous substitute is the unsatisfactory biocompatibility, especially for the synthesized polymer-based hydrogels. In this study, CCK-8 assay was performed to evaluate the cytotoxicity of the hydrogels (Fig. S2A, B). Compared to the control group (Fig. S2A1, B1), the morphology and fusion of ARPE-19 (Fig. S2A2-A4) and RF/6A (Fig. S2B2-B4) cells maintained well. Moreover, the cell viabilities of the hydrogels at low concentrations (c=9, 10 mg ml^{-1}) were all over 90% after con-culturing with the extracts for 48 h (Fig. S2C, D). In both cell lines, there was no significant difference in cell viability between the low concentration groups (c=9, 10 mg ml^{-1}) and the control group (BSS) ($P>0.05$) (Fig. S2C, 2D). However, the cell viability between the high concentration group (c=20 mg ml^{-1}) and the control group showed statistical difference ($P<0.05$) (Fig. S2C,

2D), but the relative growth rate (RGR) was also more than 70%. According to the above results, it revealed that the hydrogel with the low concentration exhibited very good biocompatibility in vitro, and further study could be implemented in live animals.

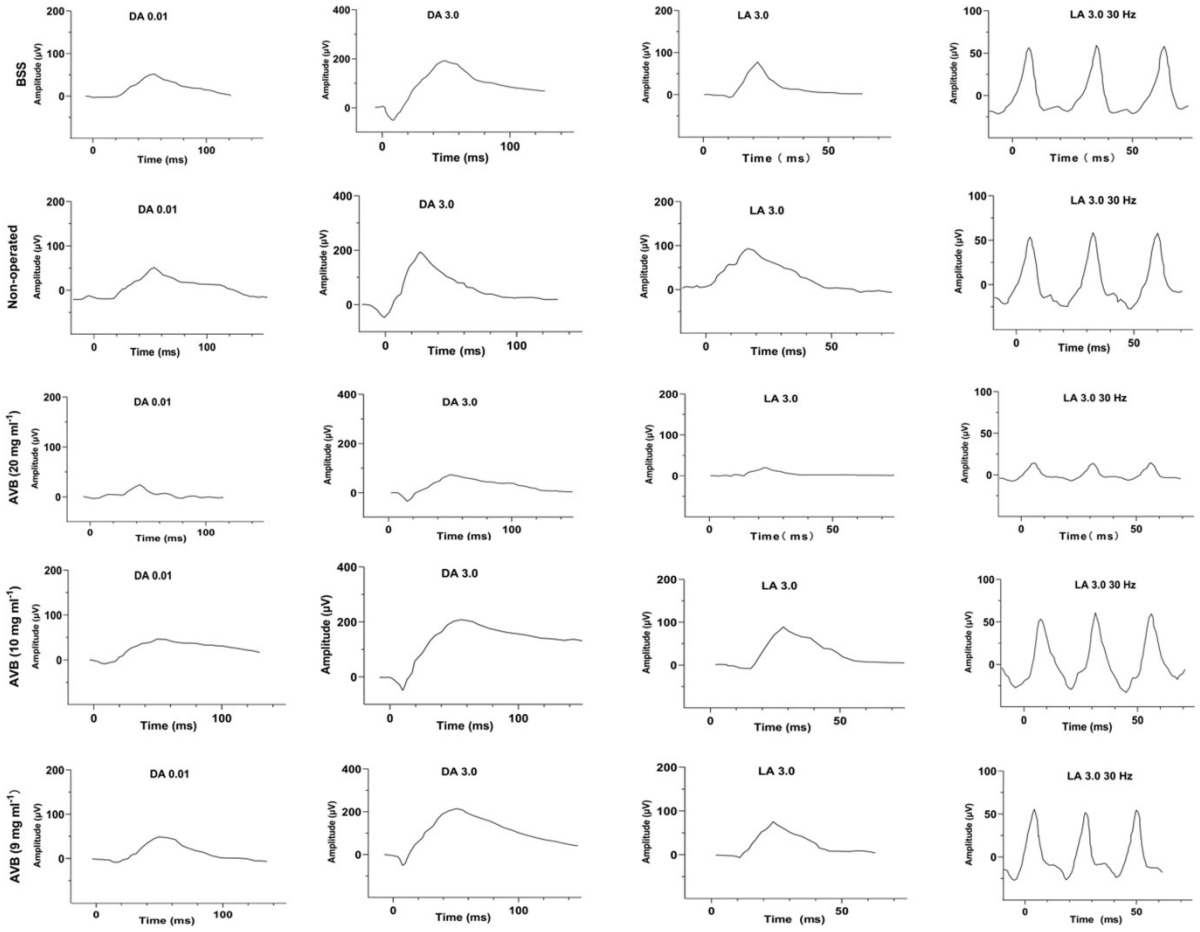

Fig. S3. Full-field ERGs were performed to assess (waveform graphs) the retinal function at 3 months after implantation of AVB.

Movie S1: The gelation times for the AVB2 when two solutions were injected into the culture dish with a double syringe through a 25G needle.

Movie S2: The appearance of the AVB2 six months after implantation in rabbit's eyes.

Movie S3: The vitreous body of the rabbit eye was removed via the 25G vitrectomy system, and then implanted with the vitreous substitutes.

References

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