Foscarnet calcium microcrystal as the intravitreal drug depot

Yuling Sun[a], Yong Tao[b,*], Qingchen Cao[a], Yanbin Huang[a,*]

[a]Key Laboratory of Advanced Materials (MOE), Department of Chemical Engineering, Tsinghua University, Beijing 100084, China

[b]Department of Ophthalmology, Beijing Chaoyang Hospital, Capital Medical University, Beijing 100020, China

*Corresponding authors: Professor Yanbin Huang (email: yanbin@tsinghua.edu.cn) and Professor Yong Tao (email: drtaoyong@sina.com)

Electronic Supporting Information

Preparation of the foscarnet calcium microcrystal

Foscarnet calcium microcrystals were synthesized via a simple salt metathesis reaction between foscarnet sodium and calcium chloride. In a typical reaction, 500 mg foscarnet sodium hexahydrate (Hubei Prosperity Galaxy Chemical Co.) was first dissolved in 250 mL deionized water, and then the solution was added into 500 mL 2 mg/mL calcium chloride dihydrate (J&K Chemicals) water solution (molar ratio of Pfa^{3-}/Ca^{2+} = 1/2, calcium ions in excess). The mixture was stirred for another hour at room temperature, and foscarnet calcium formed as precipitate. Afterwards, the stirring was stopped and the suspension was allowed to stand still for 20 min to settle down the precipitate. The supernatant was decanted, and the solid was rinsed 3 times with 500 mL deionized water and then centrifuged (6000 rmp, 10 min, TG 16-Ⅱ, Pingfan Instrument, China). The solid was collected and freeze-dried to obtain the final product for further characterizations. Foscarnet calcium microcrystals of different sizes were prepared by changing the initial concentrations of the reactant solutions.

Scanning Electron Microscope (SEM) to measure the particle size

Drops of foscarnet calcium suspension samples were transferred onto a silicon wafer, dried in vacuum oven overnight at room temperature, and then analyzed with SEM (JSM-7500F, JEOL Ltd., Japan). The particle sizes were determined using the Nano-Measure 1.2 software.

X-ray diffraction (XRD) to confirm the crystallinity

Before mounted to the XRD sample holder, the powder samples were ground with an agate mortar and sieved through a 100 mesh screen. Diffraction measurement was carried out with a Rigaku D/max-2500 X-ray diffractometer with Cu Kα radiation (1.54056 Å). Samples were placed on the sample holder and continuous scans were performed at a speed of 4° 2θ/min and 0.02° 2θ per step in the range 2θ = 5~35°.

Inductively coupled plasma optical emission spectrometry (ICP-OES) to confirm the chemical composition

Before testing, 100 mg foscarnet calcium microcrystal was fully dissolved in 2 mL nitric acid (65 wt%) and diluted with deionized water to make the final solution volume 25 mL. The concentrations of calcium, phosphorus and sodium were determined using inductively coupled plasma optical emission spectrometry (ICP-OES iCAP6300, Thermo Fisher). The

plasma power is 1150 W and the speed of peristaltic pump is 50 rmp. The atomizing flow rate and the auxiliary flow rate were 0.6 L / min and 0.5 L / min, respectively.

Thermogravimetric analysis (TGA) to check the existence of water in crystals

Before testing, samples were placed in vacuum desiccator with phosphorus pentoxide for a week in order to remove the free water. The sample was then placed in a platinum pan, and characterized using a thermogravimetric analyzer (TA Instruments TGA Q500IR). The samples were heated over the temperature range 25~1000 °C at a constant heating rate of 20 °C /min, while purged with a stream of flowing nitrogen at 50 mL/min throughout the experiment.

Solubility determination

Excess foscarnet calcium microcrystals (20 mg) were added into 10 mL different aqueous media: deionized water, 143 mM NaCl solutions with and without calcium chloride (1 mM, 3 mM, 5 mM, 7.2 mM, 10 mM). The suspension was stirred at 37 °C at 230 rmp for 48 hours. Afterwards, the suspension was centrifuged (6000 rmp, 5 min) and 4 mL supernatant was withdrawn and filtered with MF-Millipore Membranes (220 nm). The concentration of foscarnet in the solution was determined using the UV/Vis spectrophotometer with a standard curve of foscarnet sodium solution (233 nm, 1 cm light path, Abs = 0.16*C (mM)).

***In vivo* experiments**

Totally 25 healthy New Zealand white rabbits weighting 2.28 - 2.82 kg (mean weight 2.55 kg) were used in this study. The Statement for the Use of Animals in Ophthalmic and Vision Research (ARVO) was followed, and local institutional approval was received from the review committee of the Beijing Chaoyang Hospital, Capital Medical University. Pentobarbital sodium (40 mg/kg) was used to anesthetize the rabbits through intravenous injection in the ears for surgery and examinations. Before injection, the foscarnet calcium microcrystal and 1 wt% carboxymethylcellulose sodium (CMC) solution were disinfected with UV for 5 min. Then, 100 mg/mL foscarnet calcium microcrystal suspension was prepared by adding 180 mg foscarnet calcium microcrystal into 1.8 mL CMC solution, and sonicated to make it homogeneous.

The intravitreal injection was performed in the right eye of each rabbit, and the left eye was used as the control. Each rabbit was given a single intravitreal injection of 50 μL foscarnet calcium microcrystal suspension with a 29-gauge needle (inner diameter 155 μm). Ocular examinations including observation of palpebral fissure closure after light reflex were performed periodically after the intravitreal injection up to 25 weeks. At each time point of Week 1, 3, 6, 12, and 25 after the intravitreal injection, five rabbits were sacrificed with intravenous thiopentone and the eyeballs were enucleated. After the eyes were sectioned behind the lens, vitreous humor was obtained by dissecting it carefully from the retina. Vitreous samples were stored at -24 °C before testing.

Determination of the foscarnet concentration in vitreous samples

Before testing, all samples were pretreated as follows. 100 μL vitreous was mixed with 100 μL 2% trichloroacetic acid

(ANPEL Laboratory Technologies (Shanghai) Inc.) and 300 μL water. Then the mixture was centrifuged (4000 rmp, 10 min) and the supernatant was tested. Concentration of foscarnet was determined by using a high-performance liquid chromatograph (Agilent 1260 HPLC-VWD) equipped with a C18-WP column (4.6×250 mm, 5 μm) and a diode array detector. The mobile phase consisted of a mixture of 20 mM ammonium acetate (Fluka) and acetonitrile (ANPEL Laboratory Technologies (Shanghai) Inc.). The injected sample volume was 10 μL, and the flow rate of the mobile phase was 0.8 mL/min. Quantification was based on measuring standard solutions of foscarnet sodium solution and the detection limit was 0.5 μg/mL. The analysis method was validated with vitreous samples spiked with known amount of foscarnet sodium.

Electroretinogram (ERG) examination

Two rabbits (No. 24 and 25) underwent the scotopic ERG examination at Week 25 after the intravitreal injection. Briefly, the pupils were dilated with 5% tropicamide and 5% phenylephrine hydrochloride. The animals were dark adapted for at least 30 minutes. The ERG setup consisted of a contact lens electrode for each eye, a reference needle electrode positioned at the lateral canthus, and a ground disc electrode that was clipped to the earlobe of the animals. Standard ERGs were recorded (RETIport Science 4.6.0.0, Roland Consult Electrophysiological Diagnostics Systems; ROLAND CONSULT Stasche & Finger GmbH, Brandenburg, Germany). An average of six separate ERGs was used at each time point for each eye. The stimulus intensity for scotopic ERG Flashes varied in intensity from −2.5 log scot cd·s/m^2 for rod response. The b-wave was from the negative trough to the positive peak.

Histopathological studies

At Week 25 after the intravitreal injection, one rabbit (No. 24) was sacrificed and the eyeballs were enucleated and remained immersed in 4% formaldehyde for at least 24 hours at 4 °C. All tissues were embedded in paraffin, and 4 μm sections were stained with hematoxylin-eosin and photographed (IM50; Leica, Cambridge, UK).

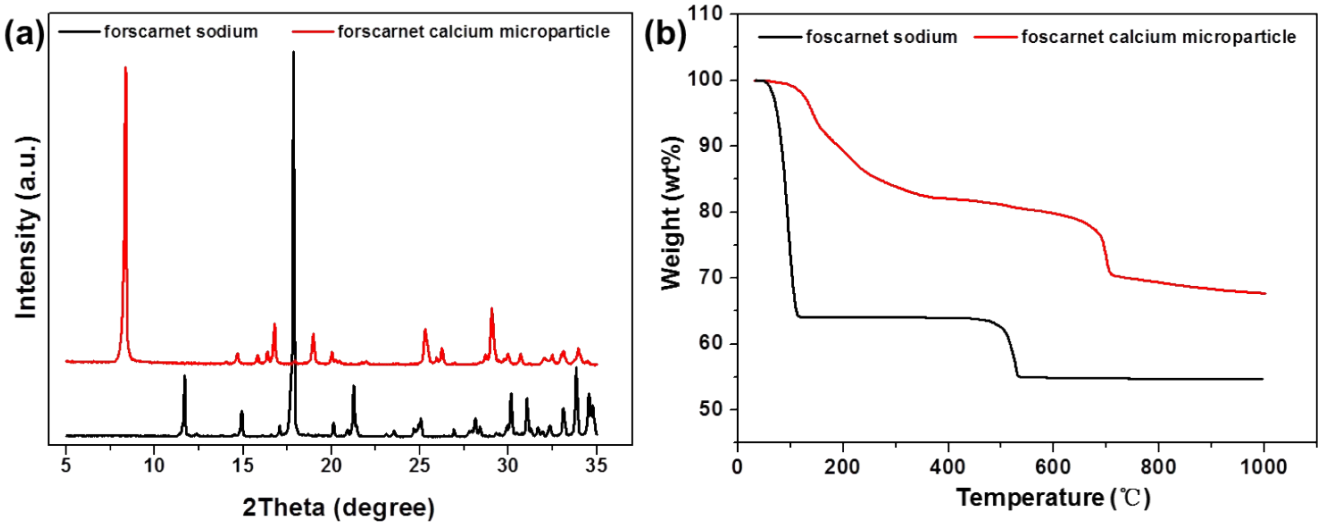

Fig. S1 (a) XRD patterns and (b) thermogravimetric analysis (TGA) thermograms of the foscarnet calcium microcrystal and the foscarnet sodium hexahydrate samples.

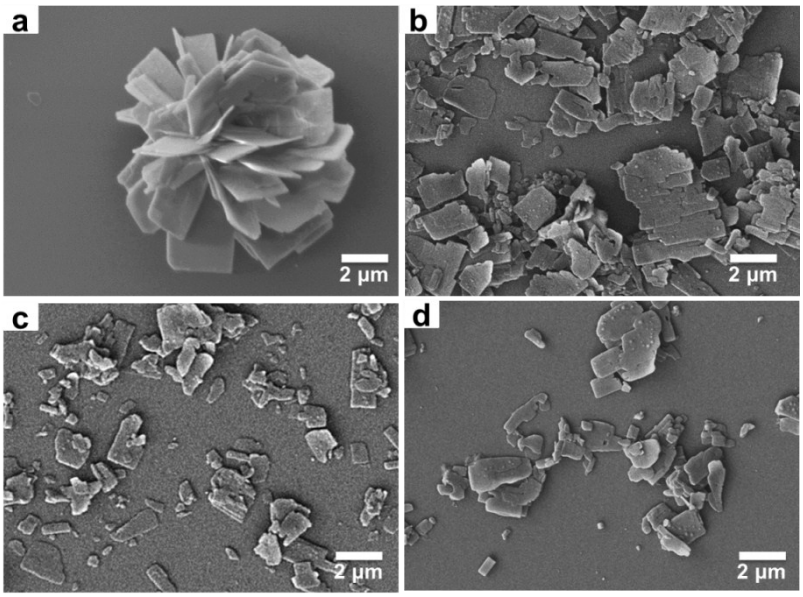

Fig. S2 SEM images of foscarnet calcium microcrystal (a) before intravitreal injection, and taken out from vitreous of rabbits at (b) Week 1, (c) Week 3 and (d) Week 12 after injection.

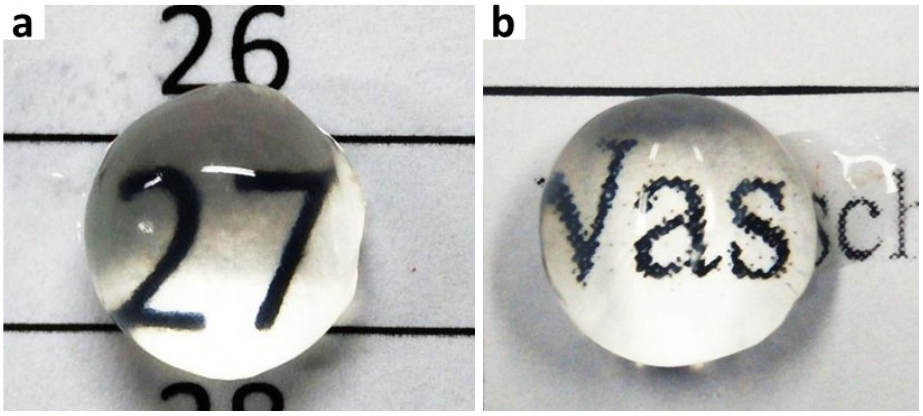

Fig. S3 The lens of rabbits at (a) week 3 and (b) week 25 after the intravitreal injection.