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https://doi.org/10.1038/s41467-024-55336-1

Wearable electrodriven switch actively delivers macromolecular drugs to fundus in non-invasive and controllable manners

Received: 9 October 2024

Accepted: 9 December 2024

Published online: 02 January 2025

Check for updates

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Current treatments for fundus disorders, such as intravitreal injections, pose risks, including infection and retinal detachment, and are limited in their ability to deliver macromolecular drugs across the blood-retinal barrier. Although non-invasive methods are safer, their delivery efficiency remains suboptimal (<5%). We have developed a wearable electrodriven switch (WES) that improves the non-invasive delivery of macromolecules to the fundus. The WES system, which integrates an electrodriven drug delivery lens with a square wave generator, leverages electrical stimulation to enhance drug penetration through the sclera-choroid-retina pathway. In our study, WES achieved a delivery efficiency of 14% for immunoglobulin G, comparable to that of intravitreal injection (16%). Moreover, WES-enhanced anti-VEGF administration resulted in an 86% inhibition of choroidal neovascularization, and anti-PDL1 delivery inhibited choroidal melanoma growth more effectively than intravenous injections, with no adverse effects on ocular health. These findings suggest that WES holds transformative potential for the non-invasive treatment of chronic fundus diseases.

Many fundus diseases, such as choroidal neovascularization and uveal melanoma, require invasive treatments because of the complex barriers within the eye. For example, choroidal neovascularization (CNV), which is often caused by wet age-related macular degeneration (AMD), trauma, and inflammation, leads to irreversible vision loss and blindness^{1,2}. Currently, anti-vascular endothelial growth factor (anti-VEGF) biomacromolecules, such as bevacizumab, are the most effective treatments for CNV, as they inhibit neovascularization^{3–5}. However, the presence of the blood-retinal barrier (BRB) necessitates invasive intravitreal injection for the delivery of these macromolecular drugs^{6–8}. On the other hand, uveal melanoma, the most common primary fundus malignancy in adults^{9,10}, often requires invasive treatments such as intraocular injections or surgical removal because of the

complex ocular barriers^{11,12}. However, repeated intravitreal injections are gloomily shadowed by the high risk of endophthalmitis infection, bulbar bleeding and retinal detachment in patients^{13,14}. These injections also have a short duration of action for small molecules and low target bioavailability for many protein- and gene-based drugs and nanomedicines¹⁵⁻¹⁷. The inner limiting membrane (ILM) also limits access to intravitreal materials into the retina⁸. Alternative methods, such as subretinal and suprachoroidal injections, are more technically demanding and less frequently used^{7,8}.

To address these challenges, non-invasive drug delivery platforms are crucial for treating multiple posterior segment ocular diseases. Although eye drops or gels based on anionic polymers, membranepenetrating peptides and exosomes have been developed as auxiliary

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delivery agents¹⁸⁻²¹, they still have limitations in enhancing the intraocular penetration of macromolecular therapeutic agents and are not conducive to clinical dissemination because of their low drug utilisation, easy clearance from the ocular surface and high systemic toxicity^{22,23}. Intriguingly, bioelectricity, a fundamental physiological signal, has shown promise in various disease interventions²⁴⁻²⁷. Exogenous electrical stimulation has significant effects on tumour treatment^{28,29}, neurological disorders³⁰⁻³², and tissue repair^{33,34}. Iontophoresis, which leverages electrophoresis for drug penetration, offers a safe, reliable, and nontoxic method for responsive drug delivery^{35,36}. Studies have demonstrated the ability of iontophoresis to enhance small molecule drug penetration in the cornea, promoting treatments for anterior segment diseases^{37,38}. However, the ability of electrical stimulation to enhance intraocular drug delivery, especially in the fundus, remains unproven.

To fill this gap, we developed a wearable electrodriven switch (WES) designed to significantly enhance non-invasive therapeutic outcomes for fundus diseases (Fig. 1a). The WES system comprised an electrodriven drug delivery lens (EDDL) and a square wave generator (SWG) (Fig. 1b and Supplementary Fig. 1). The SWG, which was embedded on a compact flexible circuit board, generated a monophasic square pulse current (PC) signal from a direct current (DC) input via an NE555-type timer chip and the corresponding circuit design (Fig. 1c and Supplementary Fig. 2). This signal was then transmitted to the EDDL, where electrodes were embedded into a polydimethylsiloxane (PDMS) lens through cast-moulding (Fig. 1d and Supplementary Fig. 3). WES-induced electrical stimulation facilitated a rapid increase of intracellular calcium ions (Ca²⁺), promoting the contraction of the perijunctional actomyosin ring (PAMR) and the redistribution of tight junction (TJ) and adherens junction (AJ) proteins in adult retinal pigment epithelial-19 (ARPE-19) cells. This mechanism significantly enhanced the delivery of macromolecular drugs to the fundus via the sclera-choroid-retina pathway. Crucially, the parameters of WES could be adjusted to control the degree and duration of cellular iunction opening (Fig. 1e).

In addition, WES-triggered iontophoresis accelerated drug distribution within the vitreous, surpassing the limitations of slow diffusion observed with intravitreal injection (Fig. 1f). The efficacy of WES has been demonstrated with various macromolecular drugs, including monoclonal antibodies, DNA origami, and extracellular vesicles. Notably, compared with intravenous injection, WES-enhanced ocular administration of anti-VEGF agents resulted in superior inhibition of CNV. Furthermore, WES-enhanced anti-programmed cell death ligand 1 (anti-PDL1) agent administration was more effective than intravenous injection in treating early-stage choroidal melanoma in mice, highlighting the platform's potential for melanoma control after excision (Fig. 1f). Long-term studies in mice confirmed the safety of WES, with no adverse effects on intraocular pressure or vision. Thus, our findings strongly supported the capacity of WES for efficient macromolecular drug delivery and its non-invasive therapeutic efficacy in treating CNV and choroidal melanoma.

The capability of WES to deliver macromolecular drugs to fundus

Previous studies have highlighted that prolonged DC stimulation can lead to adverse tissue reactions, such as Joule heating and abrupt local pH shifts²⁵. Given that the WES applies stimulation directly on the ocular surface, it is essential to confirm its cytotoxic safety profile. Therefore, we conducted comparative cytotoxicity assessments using DC and PC signals on ARPE-19 cells and human umbilical vascular endothelial cells (HUVECs), identifying safe current signal types. Our results revealed that 80 μ A DC stimulation caused significant cytotoxicity in ARPE-19 cells after 10 min, whereas 80 μ A PC (0.024 Hz) signals did not exhibit cellular toxicity (*P* = 0.0133) (Supplementary Fig. 4a). Similarly, DC caused significant cytotoxicity in HUVECs, while no toxicity under the PC treatment (P < 0.0001) (Supplementary Fig. 4a). This finding could be attributed to the fact that DC stimulation tends to induce adverse electrochemical reactions and rapid pH shifts near the electrodes, resulting in cellular damage²⁵. We further investigated the effects of PC signals generated by the SWG and highlighting the benefit of tunable frequency settings (Supplementary Fig. 4b). Furthermore, the MTT assay results confirmed the excellent cyto-compatibility of WES in ARPE-19 cells and HUVECs (Supplementary Fig. 4c, d). Moreover, WES was comfortable and safe, causing no significant temperature changes in the eye or surrounding tissues (Supplementary Fig. 5). The MTT assay results provided robust evidence that underscores the role of the SWG in converting DC to PC signals, thereby supporting the safety profile of WES.

We next used immunoglobulin G (IgG), a 150 kDa molecule like anti-VEGF and anti-PDL1, as a model drug to evaluate the ability of WES to deliver macromolecular drugs to the fundus. To facilitate visualisation, the FITC-IgG solution was applied to the Au electrode surface of the EDDL and allowed to air-dry at ambient temperature for a few minutes. This step was necessary to ensure efficient and stable attachment of FITC-IgG to the Au electrode surface. The EDDL loaded with FITC-IgG was then placed on the eyeballs of the experimental animals. By adjusting the stimulation time and frequency, we determined the optimal PC parameters. The results demonstrated that increasing the electrical stimulation time of WES significantly enhanced the delivery of FITC-IgG (Fig. 2a and Supplementary Fig. 6). Compared with EDDL, WES (0.73 Hz, 80 µA) markedly facilitated IgG delivery to the fundus with more than 5 min of WES (P = 0.0008) (Fig. 2a). Specifically, 10 minutes of WES at 0.024 Hz dramatically improved FITC-IgG penetration compared with 10 min at 0.73 Hz (P = 0.0088) (Fig. 2b and Supplementary Fig. 7). WES-enabled FITC-IgG even penetrated the retinal pigment epithelium (RPE) layer and reached the retina that strong fluorescence signals could be seen in the representative confocal images in Fig. 2d. Moreover, representative confocal fundus images from the WES group revealed stronger fluorescence signals than those from the intravitreal injection group did (P=0.0021), highlighting the efficacy of WES in non-invasive macromolecular drug delivery to the fundus (Fig. 2c, d and Supplementary Fig. 8).

We also evaluated the ability of WES to deliver various macromolecular drugs to the fundus. As depicted in Fig. 2e, f, we designed 5carboxytetramethylrhodamine-labelled rectangular DNA origami sheets (TAMRA-rDOSs) and DiD-labelled extracellular vesicles (DiD-EVs). The design and DNA Sequence of TAMRA-labelled strands was shown in supplementary information (Supplementary Fig. 9 and Supplementary Tables 1, 2). The rDOSs had extended TAMRA fluorophores at their 5'-ends, with dimensions of ~ 80 nm (Fig. 2e). The diameter of EVs was ~ 100 nm (Fig. 2f). Both the rDOSs and EVs were well stabilised, with zeta potentials of approximately - 3.5 mV and - 17 mV, respectively (Supplementary Fig. 10). The confocal images in Fig. 2g showed strong fluorescence of TAMRA-rDOSs at the choroid and retina in WEStreated eyes, whereas intravitreal injection resulted in weak fluorescence (P = 0.0039) (Fig. 2h and Supplementary Fig. 11). Similarly, WEStreated eyes exhibited strong fluorescence from DiD-EVs, unlike the intravitreal injection group (P=0.0056) (Fig. 2i, j and Supplementary Fig. 12). These results underscored the versatility of WES in delivering macromolecular drugs and nanomedicines to the fundus compared with intravitreal injection. The WES parameters of 0.024 Hz and $80 \,\mu A$ were used for subsequent experimental studies.

Quantifying the IgG concentration in WES-treated mouse eyes via enzyme-linked immunosorbent assay (ELISA) revealed a delivery efficiency of 14%, which was comparable to that of intravitreal injection at 1 h (16% of delivery efficiency), with nearly 3% detected after 24 h (Fig. 2k). Remarkably, the developed WES achieved a macromolecular drug delivery efficiency of up to 14% in fundus, which was about three



Fig. 1 | **Design of wearable electrodriven switch (WES) for enhancing macromolecular drug delivery to the fundus. a** Optical image of the EDDL used to deliver electrical stimulation to the sclera of the rabbit eye. Scale bar, 4 mm. **b** Structural diagram of the WES. The WES system consists of the EDDL and the SWG. The SWG is mounted on a flexible polyimide (PI) substrate and is responsible for generating monophasic square pulse current (PC) to facilitate electrical stimulation. The Au electrode is embedded within a polydimethylsiloxane (PDMS) lens, produced through cast moulding at ambient temperature, forming the EDDL. **c** Optical image of the WES. Scale bar, 500 μm. **d** Block illustrating the working principle of the WES. The SWG, powered by a lithium-ion battery, converts direct current (DC) into monophasic square PC signals using an NE555-type timer chip integrated into the circuit design. The frequency and duty cycle of these output signals are modifiable via a multilayer ceramic capacitor and two ceramic resistors embedded in the flexible printed circuit. The stable electrical output from the SWG is conveyed to the EDDL, which makes contact with the sclera to enable stimulation. **e**, **f** Mechanism of WES-boosted macromolecular drug delivery to the fundus. WES enhances the efficiency of macromolecular drug delivery to the fundus through the sclera-choroid-retina pathway via two main mechanisms, namely, the reversible opening of the outer blood-retinal barrier (**e**) and electrophoresis effects (**f**). MHC, major histocompatibility complex. TCR, T cell receptor. ZO-1, zonula occludens-1. F-actin, filamentous actin. Prof. Houyu Wang established the cartoons.





injection delivery, respectively (n = 3 biological replicates). Scale bar, 100 µm. **i**, **j** Representative confocal images (**i**) and statistical analyses (**j**) illustrating the distribution of DiD-EVs in the fundus following WES and intravitreal injection delivery, respectively (n = 3 biological replicates). **k** Comparative analysis of IgG delivery efficiency in mouse eyeballs after 10 min WES and intravitreal injection at 1 h and 24 h (n = 4 biological replicates). **l** Distribution of IgG in the rabbit eye choroid, cornea, and vitreous 1 h post-10 min WES and intravitreal injection delivery (n = 4 biological replicates). The data were presented as the means ± standard deviations (SDs). Error bars = SD. *P*-values in (**a**) and (**1**) were calculated via one-way ANOVA with a Tukey post-hoc test. *P*-values in (**b**), (**c**), (**h**) and (**j**) were calculated via an unpaired two-tailed *t* test. Every imaging experiment was conducted three times, yielding findings that were similar each time. *P* < 0.05 was shown. Source data are provided as a Source data file. times higher than other non-invasive methods (see Supplementary Table 3)^{15,16,21}. As a universal drug delivery platform, a delivery efficiency of 14% may enable more effective therapeutic concentrations of macromolecular drugs at the fundus, potentially improving the management of various chronic ocular diseases. This result strongly demonstrated the feasibility of WES as a non-invasive macromolecule delivery platform with significant potential for treating chronic fundus diseases. To further assess the primary distribution of IgG within the eye, we used rabbit eyes, which closely resemble the human eye structure (Fig. 2l). A higher concentration of IgG was delivered to the cornea by WES compared with intravitreal injection (P = 0.0013), probably because WES facilitated the permeation of IgG in the cornea. In contrast, only a small amount of IgG from intravitreal injection reached the cornea via the aqueous humour³⁹. However, there was no significant difference in scleral or intravitreal IgG levels between the WES and the intravitreal injection group (Fig. 2l). This result may be because the barrier properties of the ILM limit IgG permeability, resulting in lower levels of IgG entering the vitreous in the WES group and higher levels in the intravitreal injection group owing to passive diffusion³⁹. To sum up, these findings provided robust evidence that WES could significantly increase IgG delivery from the sclera to the choroid, retina and vitreous, enabling efficient non-invasive delivery of monoclonal antibodies.

The mechanism of WES to enhance macromolecular drug delivery to fundus

The TJ and AJ proteins between ARPE-19 cells formed an external blood-retinal barrier with Bruch's membrane and choroid on the outer side of the retina, limiting the movement of macromolecules⁴⁰. Therefore, opening this barrier is essential for delivering macromolecular drugs to treat fundus diseases^{41,42}. The ability of WES to reversibly open the cellular barrier was explored at the cellular and tissue levels. We first investigated the effects of WES on TJ and AJ proteins in retinal epithelial cells via WES. Experimentally, ARPE-19 cells were incubated to form a compact monolaver with closely arranged AI and TI proteins, simulating the outer blood-retinal barrier. We treated ARPE-19 cells with WES (0.024 Hz, 80 µA) for 10 min and observed the expression and distribution of TI-associated proteins. including zonula occludens-1 (ZO-1)43, E-cadherin39, occludin44, and filamentous actin (F-actin)⁴⁵, via confocal laser scanning microscopy (CLSM) (Fig. 3a). CLSM images revealed that WES triggered a redistribution of ZO-1 and occludin, especially in the regions marked by red arrows, where discrete fluorescence signals indicated open cell gaps. In contrast, untreated cells presented a continuous fluorescence grid. WES-induced redistribution of connexin ZO-1 and occludin did not significantly decrease ZO-1 and occludin expression levels. WES also caused transient E-cadherin leakage, but sufficient E-cadherin remained at the cell membrane to reform AJ and TJ. The cellular barrier was fully restored after 24 h (Third column in Fig. 3a).

Given the importance of the cytoskeleton in maintaining cell morphology and regulating TJs and AJs⁴⁶, we explored the impact of WES on F-actin expression in the cytoskeleton. Immunofluorescence (IF) images revealed dense, continuous actin in untreated cells, whereas WES-treated cells displayed F-actin breakage and leakage (Fourth column in Fig. 3a). Microscopic images in bright field of view further confirmed that the cell junctions of ARPE-19 cells were opened and substantially restored after 24 h (last column in Fig. 3a). These results suggested that WES reversibly regulated ARPE-19 cell paracellular permeability by modulating the redistribution of TJ and AJ related protein.

We also studied the transepithelial electrical resistance (TEER) of monolayer ARPE-19 cells under different WES durations (2, 5, and 10 min) with 0.024 Hz and 80 μ A (Fig. 3b). WES for 2 and 5 min immediately decreased the TEER to approximately 79% and 73%, respectively, maintaining these levels for at least one hour before

returning to 94% of the initial value within five hours. This finding indicated that short-term WES (< 5 min) could temporarily open ARPE-19 cell junctions and substantially restore them within a few hours. Ten minutes of WES prolonged junction opening, decreasing the TEER to 57 % and restoring 80 % of normal levels after 29 h (Fig. 3b and Supplementary Fig. 13). This result demonstrated WES's unique advantage of controllable junction reshaping and restoration by adjusting the WES timing.

In vivo immunofluorescence staining of mouse eye sections revealed significantly weaker fluorescence of ZO-1 in the RPE monolayer after 10 min of WES than in untreated cells (P = 0.0009) (Supplementary Fig. 14). However, the fluorescence intensity increased after 24 h and was not significantly different from that of untreated cells (Supplementary Fig. 14). These findings reinforced the conclusion that WES promoted reversible cellular junction opening of RPE monolayer.

To quantify the reversible modulation of cellular junctions by WES, we measured the paracellular permeability of a dense ARPE-19 cell monolayer to FITC-IgG. Compared with the untreated group, the WES group exhibited greater FITC-IgG permeability after 24 h (P = 0.0008), indicating enhanced paracellular junction permeability (Fig. 3c, d). Research has indicated that Rho/ROCK signalling and myosin light chain kinase (MLCK) activation synergistically phosphorylate the myosin light chain, promoting actomyosin ring contraction and junction disruption⁴⁷. Therefore, the MLCK inhibitor ML-7 was used to restore junction integrity (Fig. 3d). Notably, compared with the WES alone group, the WES + ML-7 group presented partially inhibited IgG penetration (P = 0.0003) (Fig. 3d). Our results suggested that pretreatment with the MLCK inhibitor ML-7 reversed WES-induced occludin disruption in cultured ARPE-19 cells, suggesting that WES-induced junction barrier opening is reversible (Fig. 3d).

As reported in previous literature, the extracellular repeat structural domain of E-cadherin bound Ca²⁺ to form a rigid linear molecule, which enhanced adhesion between neighbouring cells and was involved in the formation of intercellular Als^{31,47}. Therefore, extracellular Ca²⁺ depletion could contract F-actin and redistribute TI and AI proteins^{31,47}. Considering the presence of multiple calcium channels associated with electrical signalling⁴⁸, we explored the effects of WES on the free intracellular Ca2+ dynamics in ARPE-19 cells. The WES significantly remodelled the cellular junctions near the positive electrode, with reduced junction opening as the electrical potential decreased (Supplementary Fig. 15). The fluorescence intensity of intracellular Ca²⁺ was significantly stronger at the P1 position near the positive electrode than at the P_3 position away from the electrode (P = 0.0004), and the fluorescence intensity tended to decrease gradually as the distance from the electrode increased (Supplementary Fig. 16). This may be because extracellular Ca²⁺ in the near positive electrode were more susceptible to electrostatic repulsion and the cell membrane was more easily depolarised leading to a rapid increase in intracellular Ca²⁺ concentration⁴⁸. To sum up, these results confirmed the ability of WES to remodel local, controllable, and safe cellular connections. In addition, the increase in intracellular Ca^{2+} was strongly correlated with the duration of electrical stimulation of WES. WES for 2 min failed to induce free intracellular Ca²⁺ enrichment (Supplementary Fig. 17a, b). However, the intracellular Ca²⁺ concentration sharply increased after 5 min of WES (P < 0.0001), with no significant difference from that in untreated cells at 5 h (Supplementary Fig. 17c). Ten minutes of WES significantly increased the intracellular Ca²⁺ (P < 0.0001), which returned to control levels after 24 h (Fig. 3e, f). It can be found that the trend of changes in intracellular Ca²⁺ concentration at different times of WES and the changes in cellular junctions all remain almost the same. The influx of extracellular Ca²⁺ is a critical factor in regulating actomyosin contraction, which in turn controls TJs and AJs dynamics, allowing WES to modulate cellular barrier permeability in a reversible manner. These results strongly suggested that WES rapidly and



significantly regulated extracellular Ca^{2+} influx, thus enabling the reversible opening of the cellular barrier.

To explore whether WES induced an electrophoretic effect on drug delivery, we used an in vitro simulation with an agarosehyaluronic acid mixture to mimic vitreous fluid⁴⁹. In the WES group, FITC-IgG was injected into the centre of the channel and the ivoryshaped Au electrodes of SWG were attached on both sides within the channel. The Statistical analysis of mean fluorescence intensity in four regions (R_1 , R_2 , R_3 and R_4) in the pipeline after different treatments (n = 3 biological replicates per group) was shown in Fig. 3h. Each region was divided into rectangles 0.5 cm in width and 1 cm in length (direction of pipeline length). Compared with passive diffusion, WES significantly increased the fluorescence area in the channel, indicating that WES promoted the electrophoresis of FITC-IgG (P = 0.0248)

Fig. 3 | The mechanism of WES to enhance macromolecular drug delivery to fundus. a Representative immunofluorescence images illustrating the distribution of tight junction (TJ)- and adherens junction (AJ)-associated proteins, including ZO-1 (green), Occludin (green), E-Cadherin (green) and F-actin (yellow), following 10 min WES at 0 h and 24 h. Blue, DAPI-labelled cell nuclei. Scale bar, $20 \,\mu$ m. **b** Changes in the transepithelial electrical resistance (TEER) of ARPE-19 cells subjected to different durations of WES (*n* = 3 biological replicates). **c** Bar graphs showing the fluorescence intensity of FITC-IgG crossing ARPE-19 cell monolayers into the lower chamber of the Transwell system under various conditions (*n* = 3 biological replicates). **d** Comparative analysis of the fluorescence intensity of FITC-IgG in the lower chamber of the Transwell at 3 h posttreatment (*n* = 3 biological replicates). WES + ML-7: group treated with a combination of the ML-7 inhibitor and 10 min WES. **e** Representative fluorescence images of intracellular Ca²⁺ in ARPE-19 cells, as indicated by the Fluo-4 AM probe, after 0 h and 24 h of 10 min WES

(Fig. 3g, h). Finite element model analysis, as detailed in Supplementary Table 4, was used to simulate the distribution of drug concentration within the eye, demonstrating that WES-induced electrophoresis enhances drug transport compared to passive diffusion. Using COMSOL software to model the electrophoresis process triggered by WES, we identified drug charge, stimulation current, and duration as primary factors influencing drug diffusion rates. These findings highlight the distinctive advantages of the WES system for modulating drug diffusion speeds and underscore the potential for optimising electrical stimulation parameters to facilitate targeted diffusion of drugs with various charge profiles (Fig. 3i). Taken together, by triggering the extracellular Ca^{2+} influx, WES achieved reversible tight junction and adherens junction opening between retinal epithelial cells as well as cytoskeletal remodelling, which allowed macromolecular drugs to penetrate the outer blood-retinal barrier.

Therapeutic effects of WES in a laser-induced CNV mouse model

Given the efficacy of WES in the non-invasive delivery of monoclonal antibody analogues, we evaluated the therapeutic effects of WESbased delivery of anti-VEGF (aV) on CNV in mice. We established a laser-induced CNV mouse model, a well-validated model for studying exudative AMD⁵⁰ (Fig. 4a). Five days after laser treatment, fundus fluorescein angiography (FFA) revealed strong sodium fluorescein fluorescence at CNV lesion sites, and optical coherence tomography (OCT) revealed significant lesion thickening, confirming the successful establishment of the CNV model (Fig. 4b and Supplementary Fig. 18).

We divided six- to eight-week-old female C57BL/6 mice (Changzhou Cavens Laboratory Animal Co., Ltd) into five groups (n = 6 mice per group): Untreated, WES, aV, WES + aV, and aV injection. The mice in the aV injection group received a single intravitreal injection of 2 µl aV (2.4 mg/kg) on day 1 of treatment. The mice in the WES group received 10 min of WES (0.024 Hz, 80 µA) once every 4 days. The mice in the aV group were treated with aV (0.6 mg/kg) via an EDDL once every 4 days. The WES+aV group received noninvasive delivery of aV (0.6 mg/kg) via 10 minutes of WES (0.024 Hz, 80 µA) once every 4 days. After a 14-day treatment cycle, quantification of CNV lesion areas revealed that the WES+aV group most effectively inhibited new vessel growth (P < 0.0001), outperforming the intravitreal injection group (P = 0.0089) (Fig. 4b, f and Supplementary Fig. 19). OCT analysis revealed that CNV lesion thickness was significantly lower in the WES+aV group than in the untreated group (P < 0.0001), with effects comparable to those of aV injection (P < 0.0001) (Fig. 4c, g). Visual function was evaluated via electroretinography (ERG) under scotopic conditions. Statistical analysis revealed that the A and B waves in WES-treated mice were significantly restored to normal ranges, whereas those in the control groups failed to recover visual function because of inferior CNV treatment effects (P < 0.0001) (Supplementary Fig. 20). Hematoxylin treatment. Scale bar, 20 µm. **f** Statistical analysis of the mean fluorescence intensity indicating the dynamic changes in intracellular Ca²⁺ over a 24 h period after 10 min of WES (n = 4 biological independent experiments). The exact *P*-value between the Untreated and 0 h group was 3.79×10^{-9} . **g** Representative fluorescence imaging of DiD-EVs in simulated vitreous fluid treated with 10 min WES and via free diffusion. Scale bar, 5 mm. **h** Statistical analysis of mean fluorescence intensity in four regions in the pipeline after different treatments (n = 3 biological replicates). **i** Finite element model results depicting drug distribution in eyeballs delivered by 10 min WES or untreated group. The data were presented as the means ± SDs. Error bars = SD. *P*-values in (**c**, **d**, **f**) were calculated via one-way ANOVA with a Tukey post-hoc test. *P*-value in (**h**) was calculated via a paired two-tailed *t* test. Every imaging experiment was conducted three times, yielding findings that were similar each time. *P* < 0.05 was shown. Source data are provided as a Source data file.

and eosin (H&E) staining confirmed significant CNV thickness in the untreated group, whereas the WES+aV group showed significant inhibition, which was comparable to the aV injection group (Fig. 4d and Supplementary Fig. 21). IF staining of CNV-bearing mouse retinas revealed that WES + aV treatment resulted in the most pronounced reductions in VEGF and CD31 expression (Fig. 4e and Supplementary Fig. 22). Statistical analysis revealed that, compared with the other groups, the WES + aV group presented the lowest fluorescence intensities of VEGF (P = 0.0253) and CD31 (P = 0.0054) (Fig. 4h, i). These results indicated that WES could non-invasively and effectively deliver aV to the choroid and retina, demonstrating superior CNV inhibition compared with injection.

Therapeutic effects of WES in choroidal melanoma

Inspired by the promising results of WES-enhanced aV delivery in CNV therapy, we evaluated the therapeutic efficacy of WES-based anti-PDL1 (aPDL1) delivery in choroidal melanoma, validating its versatility in non-invasive drug delivery for fundus disorders. A choroidal melanoma model was constructed by microinjecting luciferase B16F10 (Luc-B16F10) cells into the subretinal chamber of six- to eight-week-old female C57BL/6 mice (Changzhou Cavens Laboratory Animal Co., Ltd) (Fig. 5a). The mice were divided into five groups (n = 5 mice per group): (G1) Untreated: Untreated groups, (G2) aPDL1: daily 10 min EDDL containing aPDL1 (0.6 mg/kg), (G3) WES: daily 10 min WES (0.024 Hz, 80 μ A), (G4) WES + aV: daily 10 min WES (0.024 Hz, 80 μ A) containing aPDL1 (0.6 mg/kg), and (G5) aPDL1 injection: aPDL1 intravenous injection (4.2 mg/kg) on day 0.

Intraperitoneal injections of D-luciferin potassium salt (10 mg/kg) followed by in vivo bioluminescence imaging at three-day intervals were used to assess treatment efficacy. The in vivo bioluminescence images of representative mice revealed that G4 had the lowest bioluminescence signal after 7 days, indicating effective tumour inhibition (P=0.0237), whereas G5 was less effective (Fig. 5b, c and Supplementary Fig. 23). Slit lamp photography on the sixth day revealed significant tumour growth in all groups except G4, which showed no tumour accentuation (Fig. 5d). Histological analysis with H&E staining revealed fewer melanomas in eye slices from the G5 group than in those from the untreated group, but melanoma cells were still present, whereas the G4 group had no melanoma cells (Fig. 5d). The survival rate was significantly prolonged to 15 days in G4 compared with the other groups (P = 0.0113) (Fig. 5e). These results confirmed that WES could cross the blood-retinal barrier, enabling effective choroidal melanoma treatment, whereas intravenous administration was hindered by ocular barrier complexities.

Subsequently, the immune response to choroidal melanoma after different treatments was investigated by flow cytometry analysis (Fig. 5f and Supplementary Fig. 24). Flow cytometry analysis revealed increased percentages of CD4⁺ and CD8⁺ T cells among CD3⁺ cells in WES-treated tumours (G4), indicating successful delivery of aPD-L1 to the tumour site and effective tumour inhibition



(*P*<0.0001) (Fig. 5g, h and Supplementary Fig. 25)⁵¹. The ELISA results revealed significant upregulation of IFN-γ in G4 compared with the other groups, and G4 had the highest levels of TNF-α (*P*<0.0001) (Fig. 5i and Supplementary Fig. 26). These results strongly confirmed that WES effectively inhibited choroidal melanoma growth by efficiently delivering aPD-L1 to tumours and activating the immune response.

In vitro and in vivo safety assessment of WES

To ascertain the safety of WES in treating fundus diseases, we conducted in vitro and in vivo evaluations over 4 weeks. Healthy six- to eight-week-old female C57BL/6 mice (Changzhou Cavens Laboratory Animal Co., Ltd) were divided into four groups (n = 6 mice per group): Untreated, EDDL, SWG, and WES. An EDDL group was included as a control to evaluate the safety of wearing the device on the ocular Fig. 4 | Therapeutic effects of WES in a laser-induced CNV mouse model.

a Diagram illustrating the construction of laser-induced CNV mouse models and various treatments. **b** Representative FFA images showing individual CNV lesions in mice before and after different treatments, with yellow circles indicating CNV lesion areas. Scale bar, $10 \,\mu$ m. **c** Representative OCT images displaying individual CNV lesions in mice before and after treatment, with the yellow rectangular box indicating CNV lesion thickness. Scale bar, $100 \,\mu$ m. **d** Representative H&E-stained retinal slices used to assess laser-induced CNV lesions in mice, with yellow circles highlighting CNV lesion thickness. Scale bar, $100 \,\mu$ m. **e** Representative IF images of VEGF (green) and CD31 (yellow) in the retinas of CNV-bearing mice (blue, DAPI-labelled cell nuclei). Scale bar, $50 \,\mu$ m. **f** Quantitative analysis of the relative CNV area before and after 14 days of treatment in five groups (n = 6 mice per group). The

surface during treatment. The EDDL consists of a PDMS lens customised for mouse eyes, integrated with a flexible gold (Au) electrode on its surface. Mice in this group wore the EDDL for 10 min per day. In addition, the SWG group was designed to assess the biosafety of the electrical stimulation parameters. In this group, electrical signals generated by the SWG were transmitted to the ocular surface via the Au electrode, with mice receiving 10-minute daily stimulations (0.024 Hz, 80 µA). For comparison, the WES group comprised mice wearing the EDDL while undergoing 10-minute daily electrical stimulation (0.024 Hz, 80 µA) from the SWG. The sodium fluorescein drops on the corneas of the WES-treated mice remained clear and unbroken, as observed via slit lamp microscopy, indicating that no corneal damage occurred after 4 weeks (Fig. 6a). Specifically, intraocular pressure (IOP) measurements at 2 and 4 weeks revealed no increase in IOP due to WES treatment compared to healthy mice in the untreated group, indicating that long-term WES induced nearly no effect to intraocular hypertension, avoiding the possibility of glaucoma (Fig. 6b). We further assessed visual function of mice after WES treatments via ERG. The representative ERG results showed that the A and B wave amplitudes in WES-treated mice were comparable to those of healthy, untreated control mice. This finding indicates that WES treatment does not negatively impact visual function, as evidenced by the preservation of normal ERG waveforms (Fig. 6c). Statistical analyses of A- and B-waves confirmed that WES did not adversely affect visual function (Fig. 6d, e). The levels of the inflammatory markers IL-1ß and TNF- α in the eyes of WES-treated mice did not indicate significant inflammation, supporting a favourable safety profile (Fig. 6f, g). The OCT images revealed no difference in retinal thickness between WEStreated and healthy mice that WES avoided negative effects on the fundus such as retinal detachment (Fig. 6h). The H&E staining revealed that the eye structures of WES-treated mice remained intact and comparable to those of healthy mice (Fig. 6i). Differences in organ structure between WES-treated and healthy mice were minimal (Supplementary Fig. 27). The blood biochemistry and blood analysis results revealed that all the parameters were within normal ranges, indicating long-term tissue safety (Supplementary Fig. 28).

Discussion

We presented a lightweight WES device for effective, non-invasive macromolecular drug delivery to the ocular fundus. Powered by a lithium battery, WES converted DC to monophasic square pulse current signals for scleral stimulation. Compelling experiment results provided substantial evidence that the brief WES periods induced a rapid increase in intracellular Ca²⁺ concentration, resulting in the remodelling of Ca²⁺-dependent cellular junctions in ARPE-19 cells. WES triggered PAMR contraction and redistributed TJ and AJ proteins, enhancing drug delivery via the sclera-choroid-retina pathway. When controlled by stimulation time and current, WES effects were localised and controllable, ensuring tissue safety. WES accelerated drug distribution in the vitreous, surpassing the slow diffusion of intravitreal injection. Our results demonstrated the superior efficiency of WES in delivering various macromolecular drugs, including monoclonal

exact *P*-value between the Untreated and WES + aV group was 6.85×10^{-7} . **g** Quantitative analysis of relative CNV thickness before and after 14 days of treatment in five groups (n = 6 mice per group). The exact *P*-value between the Untreated and WES + aV group was 3.70×10^{-9} . The exact *P*-value between the Untreated and aV injection group was 9.97×10^{-7} . **h**, **i** Quantitative analysis of the mean fluorescence intensity of VEGF (**h**) and CD31 (**i**) in five groups (n = 3 biological replicates) after 14 days of treatment. The data were presented as the means ± SDs. The error bars represent the SDs. *P*-values in (**f**-**i**) were calculated via one-way ANOVA with a Tukey post-hoc test. Every imaging experiment was conducted three times, yielding findings that were similar each time. P < 0.05 was shown. Prof. Houyu Wang established the cartoons. Source data are provided as a Source data file.

antibodies, rDOSs and EVs. WES-enhanced anti-VEGF administration effectively inhibited CNV, outperforming intravitreal injection. In addition, WES-enhanced anti-PDL1 therapy showed efficacy in non-invasive early mouse choroidal melanoma treatment. A 4-week safety study confirmed the non-invasive nature of WES and the lack of adverse effects on ocular and systemic health. This study confirmed the ability of WES to reversibly open the blood-retinal barrier via extracellular Ca²⁺ influx and junction redistribution in ARPE-19 cells. The WES platform holds promise not only for the non-invasive treatment of fundus disorders but also as a universal tool adaptable to various retinal degenerative diseases. Further research should prioritise comprehensive long-term safety evaluations of WES to ensure its suitability for chronic fundus disease treatment, a vital step for clinical translation.

Electrotherapy, employing modifiable waveforms with tunable frequency and amplitude, offers considerable promise for non-invasive, electrically mediated drug delivery systems. This study illustrates the efficacy of electrical stimulation in modulating intercellular junction permeability in a reversible and controllable manner. Future research should build on these findings to develop sophisticated, electrically responsive drug delivery platforms or therapeutic modalities rooted in electrogenetics, aiming for the effective treatment of various retinal degenerative diseases.

Moreover, prior in vitro research has substantiated that electrotherapy enhances cellular proliferation and migration while promoting stem cell self-renewal and differentiation^{24,35}. However, precise, targeted electrical stimulation at the cellular level remains pivotal for the non-invasive management of fundus diseases. For instance, future investigations should aim at embedding conductive polymers directly into affected tissues to ensure focused and efficacious stimulation, which surpasses broad, non-specific methods⁵². Furthermore, electrotherapy, when combined with stem cell therapy, has shown potential in treating neurodegenerative diseases⁵³. A significant limitation, however, is the low post-transplant survival rate of stem cells in ocular environments, which constrains the therapeutic potential of these treatments. Augmenting stem cell efficacy through the integration of electrical stimulation and engineered cellular surface modifications could substantially offer significant clinical benefits for retinal degenerative conditions⁵⁴.

Methods

Reagents and materials

N-Hydroxysuccinimide (NHS)-ester labelled fluorescein isothiocyanate (FITC) (Thermo Fisher Scientific, catalogue no. 46410), Actin-Tracker Red-Rhodamine (Beyotime, catalogue no. C2207S), Fluo-4 AM (Beyotime, catalogue no. S1061S), anti-PDL1 (Bio X Cell Biotech Co., Ltd., catalogue no. BE0101), and anti-VEGF (MCE, catalogue no. HY-P9906) antibodies were used. The rat lgG ELISA kit (Elabscience, catalogue no. E-EL-R0518), anti-occludin (Proteintech, catalogue no. 13409-1-AP), anti-E-cadherin (Proteintech, catalogue no. 20874-1-AP), anti-ZO-1 (Proteintech, catalogue no. AB0121), D-luciferin potassium salt



(Abcam, catalogue no. ab143655), ML-7 inhibitor (MCE, catalogue no. HY-15417), anti-CD3–FITC (BioLegend, catalogue no. 100204), anti-CD4–APC (BioLegend, catalogue no. 100412), anti-CD8–PE (BioLegend, catalogue no. 100708), IFN- γ ELISA Kit (Thermo Fisher Scientific, catalogue no. 88-7314-88), DAPI (Beyotime, catalogue no. C1002) and TNF- α ELISA Kit (Thermo Fisher Scientific, catalogue no. 88-7324-88) were used.

Fabrication of the WES

The SWG was obtained via a commercial flexible printed circuit fabrication process. The bill of materials includes passive components (li-ion battery, tactile switch, capacitors, resistors and diodes), two li-ion batteries (ML-621S/DN, Panasonic) that served as the power supply for the entire circuit, a tactile switch (TS2030E16055, HanElectricity) controlling the start-up and shut-down of the WES, a timer chip (NE555, IDCHIP) with **Fig. 5** | **Therapeutic effects of WES in choroidal melanoma. a** Diagram illustrating the creation of choroidal melanoma mouse models and various treatment methods. **b** Representative in vivo bioluminescence images of mice with Luc-B16F10 choroidal melanoma after different treatments. Scale bar, 4 mm. **c** Statistical analysis of average bioluminescence signal intensity in tumours over 6 days following various treatments (n = 5 mice per group). **d** Slit lamp photomicrographs and H&E staining of tumours on day 6 posttreatment, with tumours circled in yellow. Scale bar of slit lamp photomicrographs, 500 µm. Scale bar of H&E staining, 200 µm. **e** Survival rates of the mice after different treatments. **f** Representative flow cytometry analysis of CDS⁺ and CD4⁺ T cells collected from mouse eyes on day 6. **g**, **h** Statistical analysis of percentages of CD4⁺ (**g**) and CDS⁺ (**h**) T cells among CD3⁺

precision timing circuits capable of producing accurate time delays or oscillation, a multilayer ceramic capacitor (CGA0805X5R476M100MT, HRE), two switch diodes (IN4148W, YAGEO), a multilayer ceramic capacitor (0805B104K160CT-C, Walsin) and two multilayer ceramic resistors (SC0805F1304F4ANRH, Sunway) for adjusting the frequency and low-to-high ratio of the output current signals. The substrate of the flexible circuit board is polyimide (22 mm × 18 mm × 0.2 mm). The ivory-shaped flexible electrodes of the SWG were then folded and embedded in a polydimethylsiloxane (PDMS) contact lens via the cast moulding technique at room temperature to fabricate the EDDL. The connection area between the SWG and EDDL was further encapsulated by an elastomeric polyurethane coating.

Preparation of the FITC-lgG

The antibody was first prepared to a concentration of 10 mg/mL for the labelling reaction. Then the appropriate amount of NHS labelled FITC was mixed with the lgG antibody solution and ice-bathed for 2 h. Finally, the FITC labelled lgG (FITC-lgG) was obtained after removing unlabelled NHS-FITC by ultrafiltration.

Preparation of the TAMRA-rDOSs

The rDOSs were obtained by reacting the M13mp18 ssDNA scaffold (New England Biolabs, catalogue no. N4040S) with the short strand in $1 \times TAE$ -Mg²⁺ buffer and annealing according to an established procedure, which specifically consisted of heating the mixture to 65 °C and then cooling it to 25 °C at a rate of 10 min/°C. The samples were then washed twice with 400 µL of TAE-Mg²⁺ buffer and centrifuged each time for 5 min at 2000 × g. Post-centrifugation samples were analysed by using 1% agarose gel electrophoresis and an Atomic Force Microscope (AFM) (Dimension Icon, Bruker). Subsequently, an excess of TAMRA-labelled chain was added to 100 µl along with purified rDOSs. The reaction was carried out according to the established procedure: the mixture was heated to 45 °C and then cooled to room temperature at a rate of 10 min/°C. Finally, the TAMRA-rDOSs were obtained by ultrafiltration.

Preparation of the DiD-EVs

Macrophages and human retinoblastoma cell (Y79) membranes were isolated by a reported protocol. Specifically, cells were collected and resuspended in phosphate buffer (pH = 7.4) supplemented with a protease and phosphatase inhibitor cocktail consisting of 0.25 M sucrose, 1 mM EDTA, and 20 mM HEPES-NaOH. The suspensions were sonicated at 100 W with alternating cycles of 5-second cooling and 5 s shear for 10 min until a clear lysate was clear lysate was obtained. The resulting supernatant was centrifuged at $1500 \times g$ and $20,000 \times g$ for 15 min at 4 °C to remove intracellular impurities. The precipitate from the ultracentrifuge tube was then resuspended in deionised water containing 0.2 mM EDTA. Liposomes were prepared by thin film dispersion technique. DOPE, CHEMS, DSPE-PEG and DSPE-PEG-COOH in a molar ratio of 45:11.8:6:0.6 were dissolved in a chloroform/methanol mixture (9:1, v/v) and evaporated to form a thin lipid film. Ultrasound and hydration were used to generate liposomes in phosphate buffer. Then liposomes and cell membranes were combined using an extruder cells via flow cytometry (n = 5 mice per group). The exact *P*-value between the Untreated and WES + aPDL1 group in (**g**) was 2.46 × 10⁻⁶. The exact *P*-value between the Untreated and aPDL1 injection group in (**h**) was 1.72×10^{-5} . **i** Statistical analysis of IFN- γ concentrations in choroidal melanoma after different treatments via ELISA kits (n = 6 mice per group). The exact *P*-value between the Untreated and WES + aPDL1 group was 1.96×10^{-5} . The data are presented as the means ± SDs. The error bars represent the SDs. *P*-values in (**c**, **g**–**i**) were calculated via one-way ANOVA with a Tukey post-hoc test. The *P*-value in (**e**) was calculated via the Log-rank test of survival curves. Every imaging experiment was conducted three times, yielding findings that were similar each time. *P* < 0.05 was shown. Prof. Houyu Wang created the cartoons. Source data are provided as a Source data file.

(Avanti Polar Lipids, Alabaster, AL) and extruded into polycarbonate membranes. The binding ratio of liposomes to cell membranes was 1:25:25 (w/w). The morphology of EVs was characterised by the transmission electron microscope (TEM) (Tecnai G2 F20 S-TWIN, FEI). DiD-EVs were harvested by dispersing the lipid film in the DiD pre-treated phosphate buffer.

In vitro cytotoxicity analysis

The ARPE-19 cells and HUVECs were sourced from Shanghai Zhong Qiao Xin Zhou Biotechnology Co., Ltd (China) and cultured under appropriate conditions. The cell line was authenticated by species profiling and tested for mycoplasma contamination without mycoplasma contamination being found. All cell lines were cultured at 37 °C in a 5% CO₂ incubator with a humidified atmosphere. The ARPE-19 cells and HUVECs were cultured in the Dulbecco's modified Eagle's medium with high glucose (H-DMEM) which were supplemented with 10% heatinactivated foetal bovine serum (FBS) and 1% relevant antibiotics (100 µg mL⁻¹ streptomycin and 100 U mL⁻¹ penicillin). An MTT assay was performed to analyse the in vitro cytotoxicity of ARPE-19 cells and HUVECs. The cells were seeded in a 96-well plate at 5000 cells per well and incubated at 37 °C for 12 h. After different treatments and 24 h of incubation, the cells were washed and cultured with fresh media containing 5 mg/mL MTT solution. After 3 h of incubation with MTT solution, the medium was replaced with 100 µl of DMSO, and the mixture was incubated for 10 min. The absorbance at 570 nm was measured to determine the relative cell viability.

Quantitative analysis of the permeability of lgG in the eye

IgG penetration in mouse eyeballs was measured after 10 min WES or intravitreal injection at 1 and 24 h. The six- to eight-week-old female C57BL/6 mice (~20g) were acquired from the Changzhou Cavens Laboratory Animal Co., Ltd. Mice were housed in groups of 5 mice per cage and maintained at a temperature of ~25 °C in a humiditycontrolled environment with a 12 h light/dark cycle. After the experimental mice were euthanised, the eyeballs were collected and cleaned with a buffer solution. Supernatants from homogenised eyeball tissue were collected after centrifugation and detected via ELISA kits. The eight-month-old New Zealand rabbits were acquired from the Shanghai Jiagan Biological Technology Co., Ltd. Rabbits were housed in groups of 2 rabbits per cage and maintained at a temperature of ~25 °C in a humidity-controlled environment with a 12 h light/dark cycle. The IgG distribution in New Zealand white rabbit eyeballs was measured after 10 min WES or intravitreal injection at 1 h. The rabbit eyeballs were washed and dissected into the cornea, choroid, and vitreous after treatment, and the IgG concentration was measured via ELISA kits.

Immunofluorescence analysis of tight junction and adherens junction proteins in ARPE-19 cells and eyeballs

The ARPE-19 cells were seeded on a 12-well plate at $5 \times 10^{\circ}$ cells per well and incubated at $37 \,^{\circ}$ C for 24 hours. Cells and mouse eyeballs, untreated or treated with WES for 10 minutes, were fixed in 4 % paraformaldehyde at $37 \,^{\circ}$ C for 15 min and washed with PBS. The samples were permeabilized with 0.5 % Triton X-100 in PBS at $37 \,^{\circ}$ C for 20 min,



Fig. 6 | **In vitro and in vivo safety assessment of WES. a** Representative images of fluorescein cornea staining and slit lamp microscopy in different groups over one month. Scale bar, 500 μ m. **b** Statistical analysis of intraocular pressures (IOPs) in mouse eyes after 4 weeks of various treatments (*n* = 6 mice per group).

c–**e** Representative electroretinography (ERG) recordings (**c**) and statistical analysis of A-wave (**d**) and B-wave (**e**) amplitudes across the four groups after one month (n = 6 mice per group). **f**, **g** Quantitative analysis of the inflammatory cytokines IL-1 β

(f) and TNF- α (g) in the eyes of each group after 4 weeks (n = 6 mice per group). *P*-values in (f) and (g) were calculated via one-way ANOVA with a Tukey post-hoc test. **h** Representative FFA and OCT images of the groups after 4 weeks, with the FFA scale bar at 10 μ m and the OCT scale bar at 100 μ m. **i** Representative H&E-stained slices of eyeballs collected from mice in different groups. Scale bar, 200 μ m. The data were presented as the means ± SDs. All imaging experiments were repeated three times with similar results. Source data are provided as a Source data file. washed with PBS, blocked with 5 % bovine serum albumin in PBS at 37 °C for 30 min, and incubated with primary antibodies (anti-ZO-1 (Proteintech, catalogue no. 21773-1-AP, 1:1000), anti-E-cadherin (Proteintech, catalogue no. 20874-1-AP, 1:500)) at 4 °C overnight. The samples were then incubated with FITC-conjugated secondary antibodies (Abways, catalogue no. AB0121, 1:1000) at 37 °C for 2 h, stained with actin-tracker red-rhodamine (Beyotime, catalogue no. C2207S, 1:200) and DAPI (Beyotime, C1002, 1:1000), and imaged via an LSM 800 confocal laser scanning microscope.

TEER measurement of the ARPE-19 cells

ARPE-19 cells were seeded at 5×10^4 cells per well in the top chamber of a transwell to form a dense monolayer barrier. The cells were treated with WES for 2, 5, or 10 min, and the TEER values were measured over 30 h.

TEER (ohm
$$\cdot$$
 cm²) = (R₁ - R₀) × 0.33

where R_1 is the TEER value with cells and R_0 is without cells. The top chamber membrane area was 0.33 cm².

Quantitative analysis of the permeability of FITC-lgG in monolayers of ARPE-19 cells

ARPE-19 cells were seeded at 5×10^4 cells per well in the top chamber of a transwell. FITC-IgG was added to the top chamber, and the samples were treated via different methods. In the ML-7 group, the cells were pretreated with the ML-7 inhibitor overnight. In the WES group, the cells were treated with WES for 10 min. In the WES + ML-7 group, the cells were treated with WES for 10 min after pretreatment with the ML-7 inhibitor overnight. The fluorescence intensity of FITC-IgG was measured via a fluorescence spectrometer.

Intracellular Ca²⁺ imaging during WES

ARPE-19 cells were seeded on a 6-well plate at 2×10^6 cells per well and incubated at 37 °C for 12 h. A total of 1 mL of Fluo-4 staining solution (assay buffer with 0.2 % Fluo-4 AM and 0.2 % solubility enhancer) was added to each well, and the mixture was incubated at 37 °C for 30 min. The cells were treated with WES for different durations, and Fluo-4 AM fluorescence was imaged via an LSM 800 confocal laser scanning microscope.

In vitro electrophoretic ability analysis of WES

A solution containing 0.95 mg/mL agarose and 0.7 mg/mL hyaluronic acid was used to simulate vitreous fluid. DiD-EVs were used as large model drugs with WES for 10 min. Fluorescence images and statistical analysis of the mean fluorescence intensity of DiD-EVs were obtained via an animal-living fluorescence imaging system.

Theoretical simulation of the potential distribution in cell culture dishes under WES

Theoretical simulations of the potential distribution in cell culture dishes under WES were conducted via COMSOL Multiphysics software with current modules⁵⁵. A constant current formed a stable potential distribution in the cell culture dish. Current module equations:

$$\nabla \cdot J = Q_{j,v} \tag{1}$$

$$J = \sigma E + \frac{\delta D}{\delta t} + J_{\rm e} \tag{2}$$

$$E = -\nabla \cdot V \tag{3}$$

$$V = V_0$$

where *V* denotes the electrical potential, *E* refers to the intensity of the electric field, *J* represents the current density, σ represents the material conductivity ∇ and represents the Hamiltonian.

Theoretical simulations of WES-facilitated drug electrophoresis Theoretical simulations of the WES-facilitated drug electrophoresis were conducted via COMSOL Multiphysics software with current modules and substance transmission modules⁵⁶. The electrophoretic effect of WES on the drug diffusion was visualised by constructing a simplified 2D eye model emulating the experimental device layout. Two Au electrodes loading with the drug are symmetrically distributed on both sides of the eyeball surface, one of which was labelled as the ground. The vitreous fluid was modelled as the eye's fluid environment, with WES in contact with the scleral surface. The dynamic substance penetration process was then simulated by the substance transmission module on the basis of the current module. The theoretical equation can be expressed as follows:

$$\frac{\partial c_{i}}{\partial t} + \nabla \cdot J_{i} = R_{i}$$
(5)

$$J_{i} = -D_{i}\nabla c_{i} - z_{i}u_{m,i}Fc_{i}\nabla V$$
(6)

where J_i refers to the diffusion flux vector, c denotes the concentration, z represents the charge number, F refers to the Faraday constant, V is the electrical potential, D_i corresponds to the effective diffusion coefficient, and $u_{m,i}$ denotes the effective mobility. The relationship between D_i and $u_{m,i}$ can be expressed via the Nernst–Einstein equation:

$$u_{\rm m,i} = \frac{D_{\rm i}}{RT} \tag{7}$$

where R represents the Moore gas constant, and T represents the temperature.

The therapeutic effects of WES in CNV mice

A mouse model of laser-induced CNV was established via laser photocoagulation (370 mW, 60 ms) according to a standard protocol⁴⁸. CNV lesions were observed via an OCT system (Heidelberg, Germany) after the intraperitoneal injection of 2 % fluorescein sodium (Alcon) (5 mL/kg). The mice were divided into five groups: Untreated, WES, aV, WES + aV, and aV injection. The CNV lesion area and thickness and the mean fluorescence intensity of VEGF and CD31 were calculated via ImageJ software.

The therapeutic effects of WES in choroidal melanoma mice

The choroidal melanoma model was created by injecting 2×10^5 Luc-B16F10 cells into the subretinal chamber of C57BL/6 mice. After 2 days, the mice were divided into five groups: (G1) Untreated, (G2) 10 min EDDL containing aPDL1 (0.6 mg/kg) daily, (G3) 10 min WES daily, (G4) 10 min WES containing aPDL1 (0.6 mg/kg) daily, and (G5) aPDL1 intravenous injections (4.2 mg/kg) on day 0. The mice were injected intraperitoneally with D-luciferin potassium salt (10 mg/kg) and imaged via an in vivo bioluminescence imaging system at three-day intervals. Immune cells in choroidal melanoma tumours were studied by obtaining single-cell suspensions from mouse eyes at 6 days posttreatment and then stained with anti-CD3–FITC, anti-CD4–APC, and anti-CD8–PE antibodies. IFN- γ and TNF- α concentrations were measured via ELISA kits. Eyeballs were sectioned (8 µm thickness), stained with H&E, and imaged via light microscopy (DM 4000).

Statistical analysis

(4)

A two-tailed *t* test was employed for comparisons between two groups, while a one-way analysis of variance (ANOVA) with a Tukey post-hoc test was conducted for multiple group comparisons using GraphPad software. All the data are presented as the means \pm SDs. The quantitative assessment of fluorescence intensity was performed via ImageJ, a commercial image analysis programme.

Ethics

All the animal experiments were conducted in accordance with the animal experiment protocols approved by the Soochow University Laboratory Animal Centre (approval number: syxk (su) 2021-0073). The animal experiments were approved by the animal welfare regulatory authorities.

Reporting summary

Further information on research design is available in the Nature Portfolio Reporting Summary linked to this article.

Data availability

All data supporting the findings of this study are available within the article and its supplementary files. Any additional requests for information can be directed to and will be fulfilled by, the corresponding authors. Source data are provided in this paper.

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Acknowledgements

We thank Prof. Qian Chen (Sochoow University, China) for her general help and valuable suggestions. Y.H. discloses support for the research described in this study from the National Key R&D Programme of China (2023YFB3208200), the National Natural Science Foundation of China [grant numbers 22393932, T2321005, 21825402], the Science and Technology Development Fund, Macau SAR [grant number 0002/2022/ AKP, 0115/2023/RIA2] and the Programme for Jiangsu Specially Appointed Professors to Professor Yao He, a project funded by the Priority Academic Programme Development of Jiangsu Higher Education Institutions (PAPD), 111 Project and Collaborative Innovation Centre of Suzhou Nano Science and Technology (NANO-CIC). H.Y.W. provided support for the research described in this study from the National Natural Science Foundation of China [grant number 22074101]. X.H.S. provided support for the research described in this study from the the Major Independent Research Project of Jiangsu Key Laboratory for Carbon-Based Functional Materials & Devices (grant number L421490022). Z.W. provided support for the research described in this study from the National Natural Science Foundation of China (No. 62174115, No. U21A20147), the Natural Science Foundation of Jiangsu Province of China (No. BK20240152), the Natural Science Foundation of Jiangsu Higher Education Institutions of China (No. 24KJA430011). B.B.C. provided support for the research described in this study from the National Natural Science Foundation of China (No.

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X.Q., B.B.C., Z.W., X.H.S., H.Y.W. and Y.H. conceived and designed the research. X.Q., H.L.S. and H.Y.L. carried out most of the experiments and analysed the data. J.W.Z. performed additional experiments and characterisations. X.Q., H.L.S., Z.W., X.H.S., H.Y.W. and Y.H. wrote the manuscript.

Competing interests

The authors declare no competing interests.

Additional information

Supplementary information The online version contains supplementary material available at https://doi.org/10.1038/s41467-024-55336-1.

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Peer review information *Nature Communications* thanks Yang-Bao Miao, and the other anonymous reviewer(s) for their contribution to the peer review of this work. A peer review file is available.

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