



Investigating retinal endovascular chemotherapy for retinoblastoma

Jared Ching^{1,2}, Shohei Kitahata¹, Chen Sensei³, Sota Ogura³, Shigenobu Suzuki³, Kazuaki Kadonosono¹
¹Department of Ophthalmology and Microtechnology, Yokohama City University Medical Centre, Yokohama, Japan
²Department of Engineering Science, University of Oxford, Oxford, United Kingdom
³Department of Ophthalmic Oncology, National Cancer Center Hospital, Tokyo, Japan

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ABSTRACT

Background: Current chemotherapy delivery routes for retinoblastoma include intravenous, intra-arterial, intravitreal, and intracameral administration. Despite these options, recurrence can occur, highlighting the need for alternative strategies. Direct transvitreal intravascular delivery has not yet been explored. We investigated the feasibility of using 49-gauge (49G) microneedles to deliver melphalan directly into the retinal vasculature.

Methods: Y79 retinoblastoma cells were exposed to increasing concentrations of clinical-grade melphalan (0.1–10 µg/mL). Cell viability was assessed using XTT and SYTOX assays, comparing delivery by microneedle with standard pipetting. For ex vivo validation, fluorescent microbeads mixed with melphalan were injected into porcine retinal vessels using a closed-globe vitrectomy setup.

Results: Melphalan produced a dose-dependent reduction in Y79 cell viability. Microneedle delivery was non-inferior to pipette delivery across concentrations tested. Ex vivo experiments confirmed successful intravascular delivery, with fluorescent microbeads detectable in distal vessels and confined to the vascular lumen without extravasation.

Conclusions: This proof-of-concept study demonstrates the feasibility of ultra-fine microneedle-mediated intravascular delivery of chemotherapy to the retina. Further refinement of this microinvasive approach is warranted to evaluate its translational potential in retinoblastoma treatment.

AIM

Assess the flow and efficacy of clinical grade melphalan delivered by microneedles compared to controls and confirm microsurgical feasibility with an ex vivo model.

METHODS

Melphalan preparation
Alkeran 50mg (Sandoz, Basel, Switzerland) as used in patients intra-arterial chemotherapy .

Fluorescent Microbeads
Fluorescent polystyrene microspheres, Suncoast yellow were prepared as a 1:100 in melphalan for the purposes of fluid tracing in an ex vivo eye model.

Cell culture
Human retinoblastoma Y79 (ATCC, USA) cell line was used for cell culture, cell viability assays SYTOX and and MTT assay (Fig. 1A).

Surgical instrumentation
Constellation Vision System (Alcon, Fort Worth, TX, USA) was used. Tochigi Seiko 0.05mm microneedle. All experiments carried out in vitro utilized a HEPA filtered sterile hood alongside sterile surgical instruments.

Ex vivo retinal endovascular chemotherapy delivery
Freshly harvested 3-6 month old pig eyes within 24 hours.

Results

In vitro

- No significant difference between the control and microneedle groups in terms of mean cell death (Fig. 1B-D), although the raw figures suggested that the microneedle delivered melphalan tended towards a higher degree of cytotoxicity (Fig. 1C).
- At the highest melphalan concentration of 10µg/mL, mean (standard deviation) cell death was found to be 17.47% (±7.75%) and 38% (±6.26), p = 0.068, delivered with the control pipette and microneedle, respectively.
- Mean cell viability measured with MTT assay found no statistically significant difference between the control pipette and microneedle (Fig. 1D). At the highest concentration of melphalan of 10µg/mL, cell viability was 25.96% (±21.86%) and 33.08% (±47.46%) for the control pipette and microneedle, with no statistically significant difference, p = 0.78. Using 2.5µg/mL of melphalan, the mean cell viability was lower than 5.0µg/mL and 10µg/mL with 5.33% (±28.81%) and 12.27% (±42.34%) for the control pipette and microneedle, with no statistically significant difference, p = 0.58.

Ex vivo
All retinal vascular cannulations were successful and verified that melphalan was found in downstream retinal vessels at 2mm (Fig. 2).

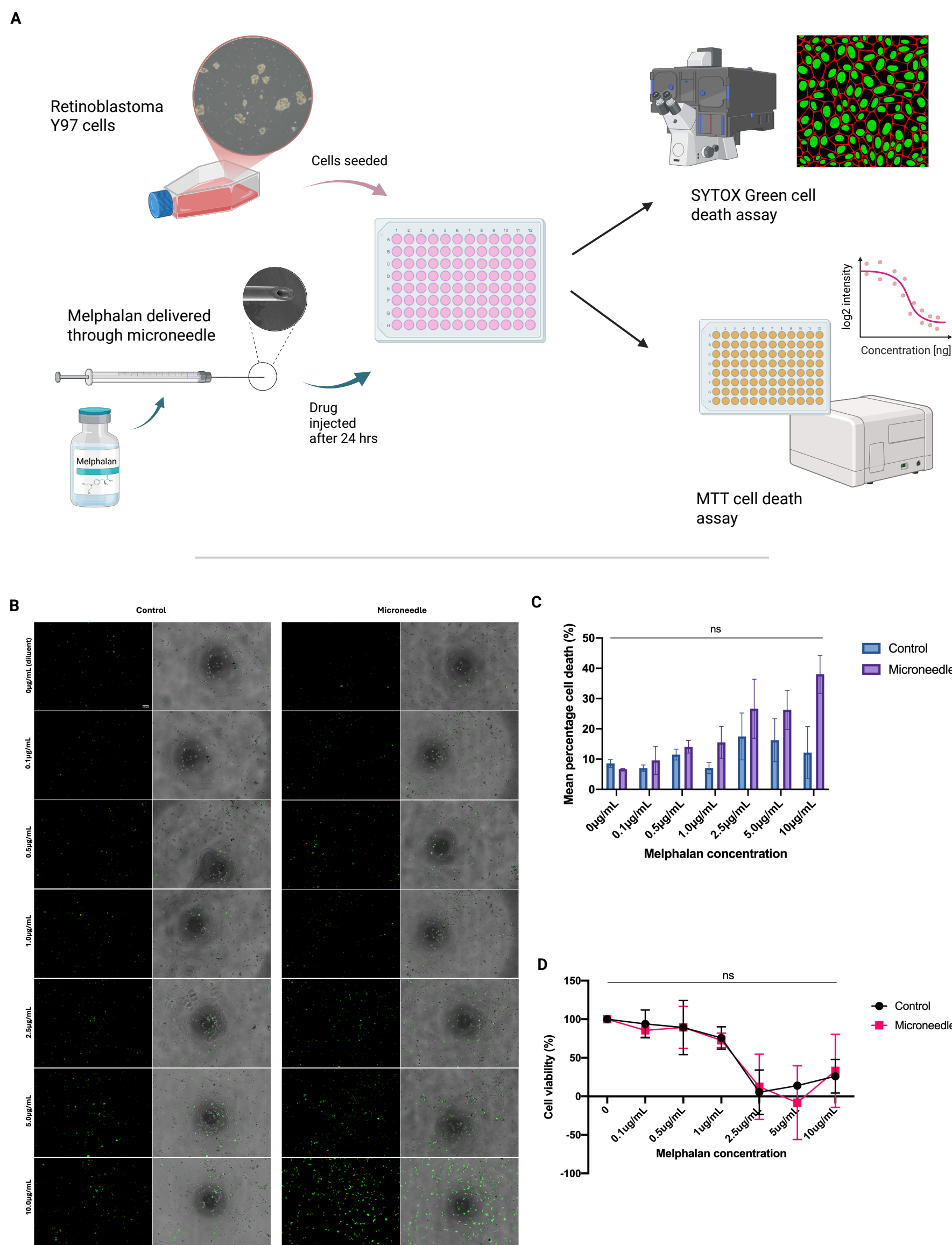


Figure 1.: Summary of comparison of melphalan delivery through a microneedle compared to control in vitro. Schematic showing sequence of experiment whereby Y79 retinoblastoma cells are seeded in a 96 well plate and then increasing concentrations of melphalan are added from 0µg/mL (diluent) to 10µg/mL, followed by viability assays using SYTOX green and MTT assay (A). Fluorescent and merge with phase contrast images for the control and microneedles using SYTOX green, where the scale bar indicates 100µm and applies to all images in this panel (B). Mean cell viability using SYTOX green at different melphalan concentrations after 24 hours of culture (C). MTT assay results comparing cell viability using the control and microneedles (D).

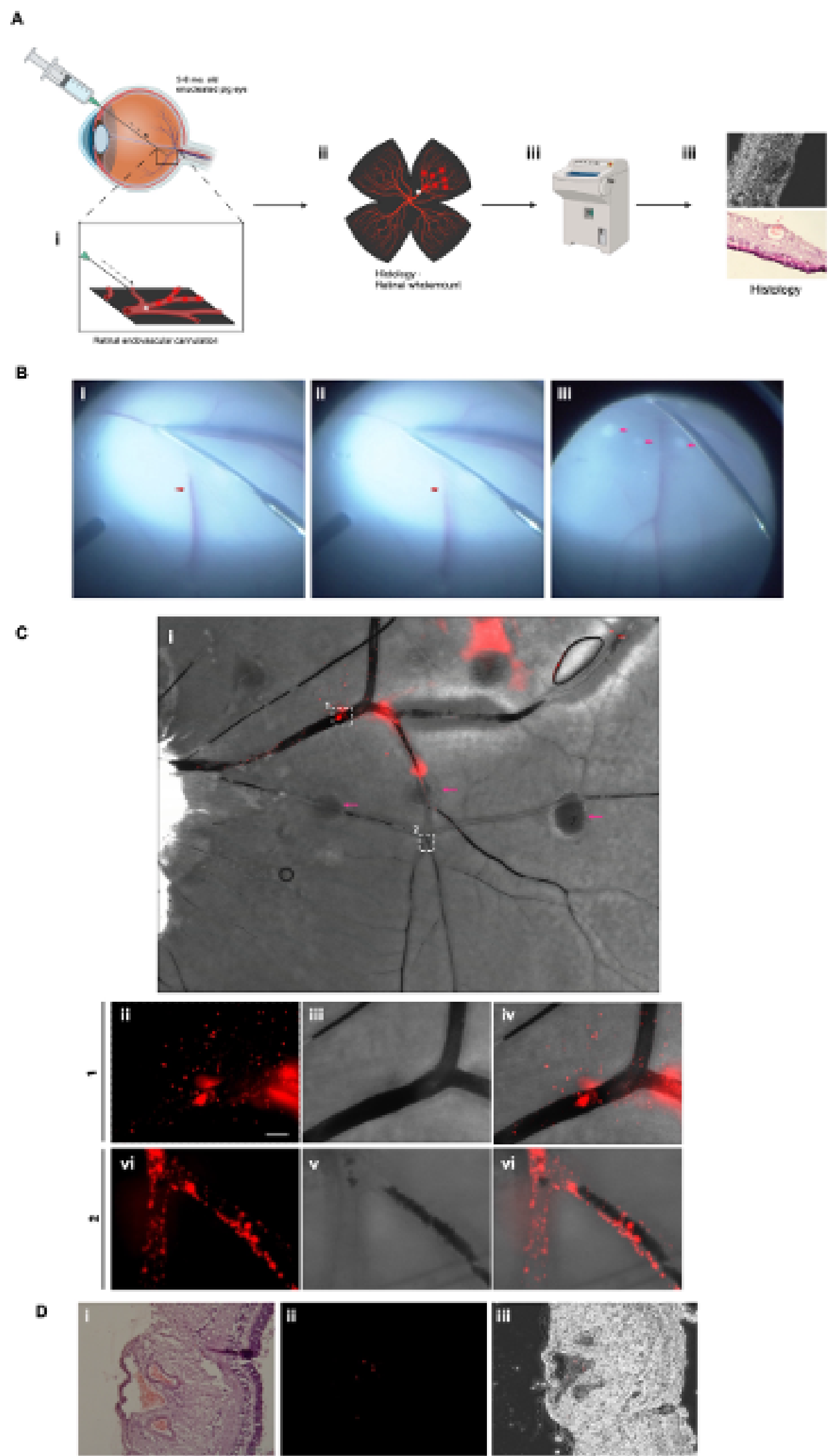


Figure 2. Summary of retinal endovascular delivery of a melphalan-fluorescent microbead solution in a porcine ex vivo model. (A) Schematic summarizing surgical delivery using a microneedle via a three port 25 gauge pars plana vitrectomy approach by (i) cannulating retinal vessels and injecting the melphalan-fluorescent microbead solution, where the asterisks indicate the injection site, followed by tissue fixation and preparation of a (ii) retinal wholemount for tracing of the melphalan-fluorescent microbead solution. (iii) Targeted cryosectioning to identify fluorescent microbeads within the vascular walls was then undertaken with confirmation of the presence of blood cells to validate that a blood vessel has been cannulated and injected accurately using hematoxylin and eosin staining. (B) Intraoperative images during surgery of a fresh ex vivo eye showing (i) microneedle cannulation of a retinal vessel, (ii) blushing of the coagulated blood in a distal branch vessel (red arrows) during the melphalan-fluorescent microbead injection, and (iii) retinal diathermy marking, pink arrows, to allow accurate isolation of the injection site for further histological analysis. (C) Fluorescent microscopy images demonstrating (i) retinal wholemount with fluorescent microbeads (red) in areas of posterior cortical vitreous and intravascularly. Pink arrows correspond to diathermy marks as shown in Biii. (C) Zoom images of the injection site: (ii) red channel, (iii) phase contrast, (iv) merge, showing foci of red hyperfluorescence of the microneedle injection site. (v) Downstream vascular bifurcation shows the presence of the melphalan-fluorescent microbead solution distributed within the vascular lumen only. (D) Cross sectional images confirming the (i) presence of red blood cells within the vessel using hematoxylin and eosin staining, (ii) red channel fluorescent microscopy and (iii) merge images with phase contrast, showing that the fluorescent microbeads are distributed within the vessel walls in cross section. Scale bar in Cii-vi represents 100µm and applies to Cii-vi and Dii-iii.

CONCLUSIONS

It is feasible to deliver melphalan chemotherapy through a microneedle. We demonstrate proof of concept with an ex vivo model, providing the impetus to carry this forward into animal studies with the hope of translating this into an additional treatment modality for managing globe salvage in retinoblastoma in the future.