

INTRODUCTION

Subretinal delivery of ocular gene therapy products has emerged as the therapeutic strategy for patients with previously untreatable inherited retinal diseases. Compared to other ocular routes of administration, subretinal injection allows for targeted delivery of the therapeutic agent to the subretinal space. Despite these exciting advances in viral vector delivery to the subretinal space, a review of the literature reveals limited analysis of the potential ocular toxicity of subretinal injections in cynomolgus monkeys. This poster aims to summarize and review ocular findings from preclinical toxicology studies of subretinal gene therapy in cynomolgus monkeys, with the intention of serving as a reference for future non-clinical safety evaluations and clinical studies.

METHODS

A total of 180 adult cynomolgus monkeys (3 to 5 years old, F:M=1:1) were dosed with different ocular gene products. The serotypes of the vector of gene therapy products are adeno-associated virus (AAV) 2, 5, 8 and 9, with potential indications of Bietti crystalline dystrophy (BCD), Leber's congenital amaurosis (LCA), and X-linked recessive retinitis pigmentosa (XLRP). Subretinal injections were performed between upper and lower vascular arches with injection volumes ranging from 50 μ L to 100 μ L, at different dose levels (1.5E10 -2.4E12 vector genomes (vg)/eye). Fifty of 180 animals were dosed with virus diluent (placebo) or empty vector as controls. Fundus photography (FP), fundus fluorescein angiography (FFA), optical coherence tomography (OCT), and electroretinogram (ERG) were used to assess changes of retinal structure and function at 2, 4, and 13 weeks after injection.

RESULTS

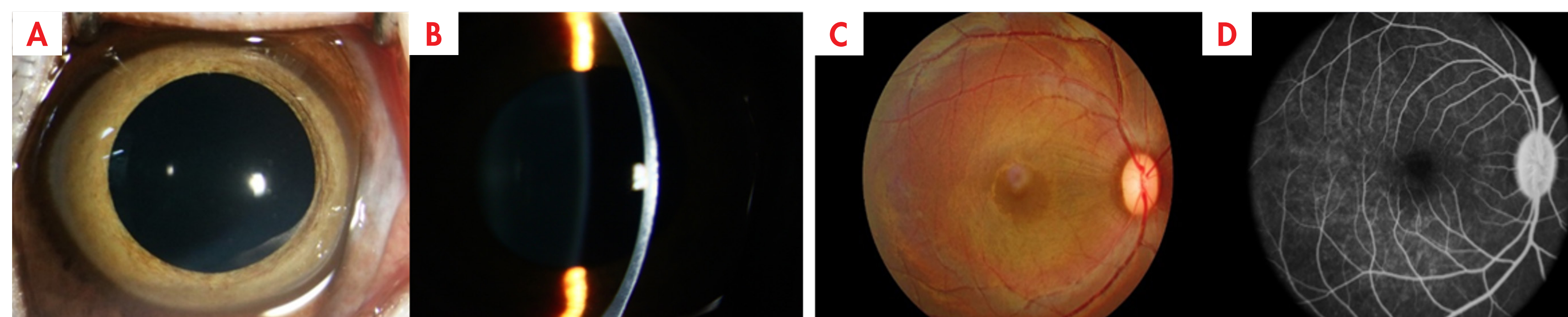


Figure 1. Before subretinal injection, all animals underwent a comprehensive ophthalmic examination including slit-lamp examination (A and B), FP (C), FFA (D), OCT (E and F) and ERG. No abnormal findings were observed.

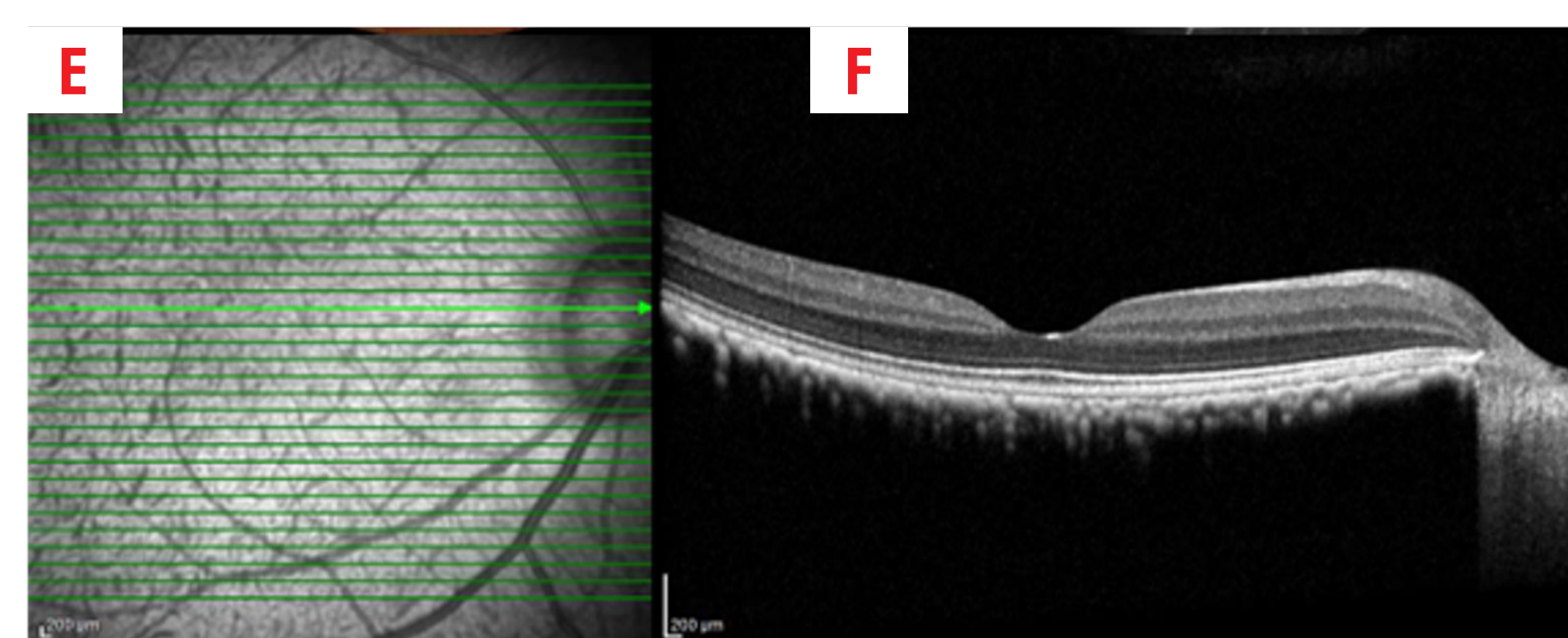


Figure 2. Right after injection, retinal detachment with well-formed bleed containing drug solution can be observed by FP and OCT in the injection area. The size is determined by the injection volume (100, 70, 50 μ L, from top to bottom). The blue arrow indicates mechanical damage at the needle insertion site, with or without slight hemorrhage.

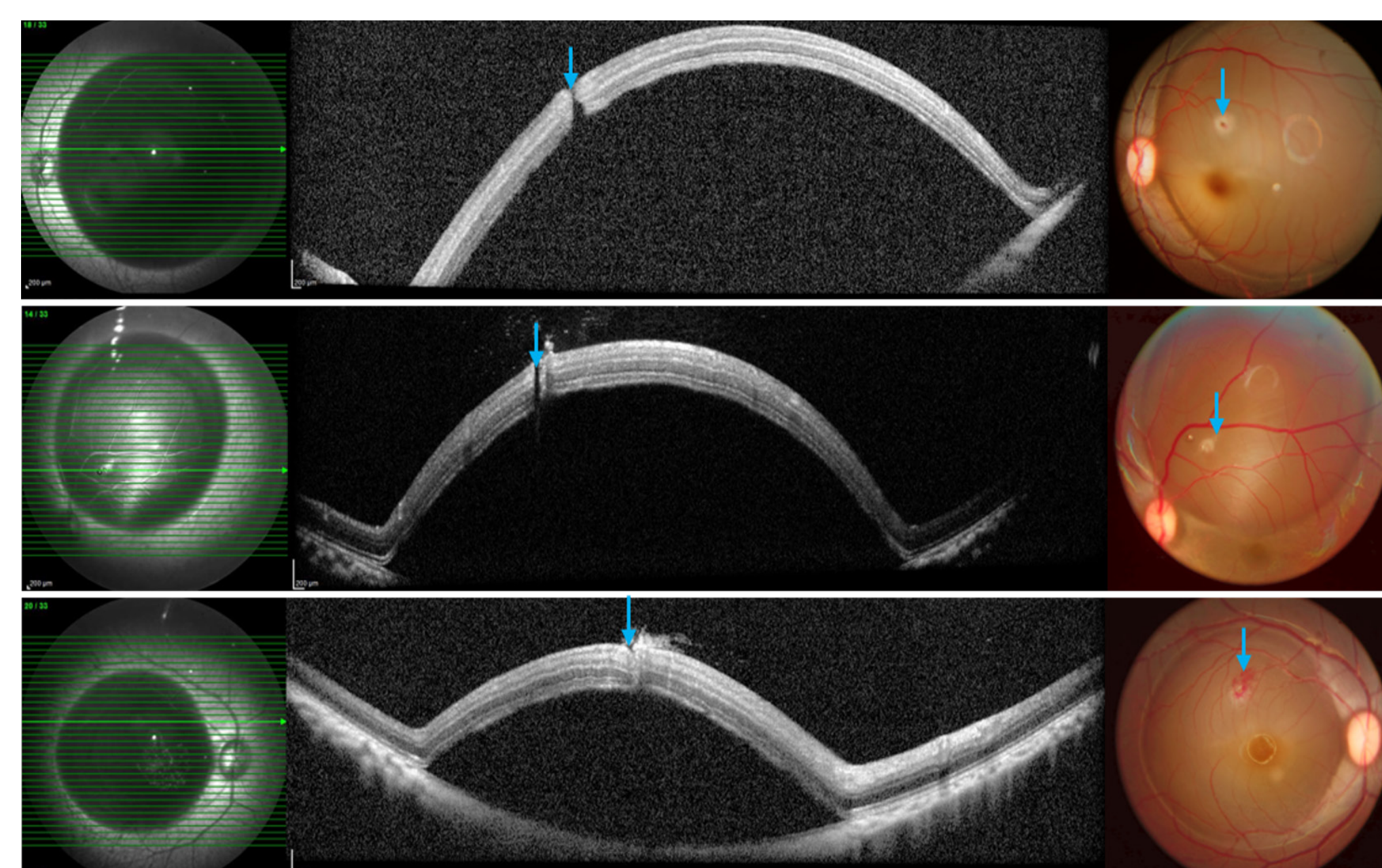


Figure 3. Incomplete absorption of subretinal blebs within 2 weeks post dosing (A) and fully reattachment of retina at 2 weeks or later post dosing (B). The red box indicates the injection area.



Figure 4. In the eyes injected with control solution, at 2 weeks post dosing, retinal depigmentation was observed at the center of the injection site, which was manifested as mottled transparent fluorescence (window defect) in FFA and correlated with mild disorganization and thinning of RPE in the OCT scan at the corresponding area (indicated by the green arrows). At the boundary of injection site, obvious pigmentation can be observed in FP, which correlates with the hypo-fluorescence in FFA and the thickening of retinal pigment epithelial (RPE) in OCT scans (indicated by the blue arrows). These findings were present from 2 weeks after dosing with a recovery trend at 13 weeks after dosing.

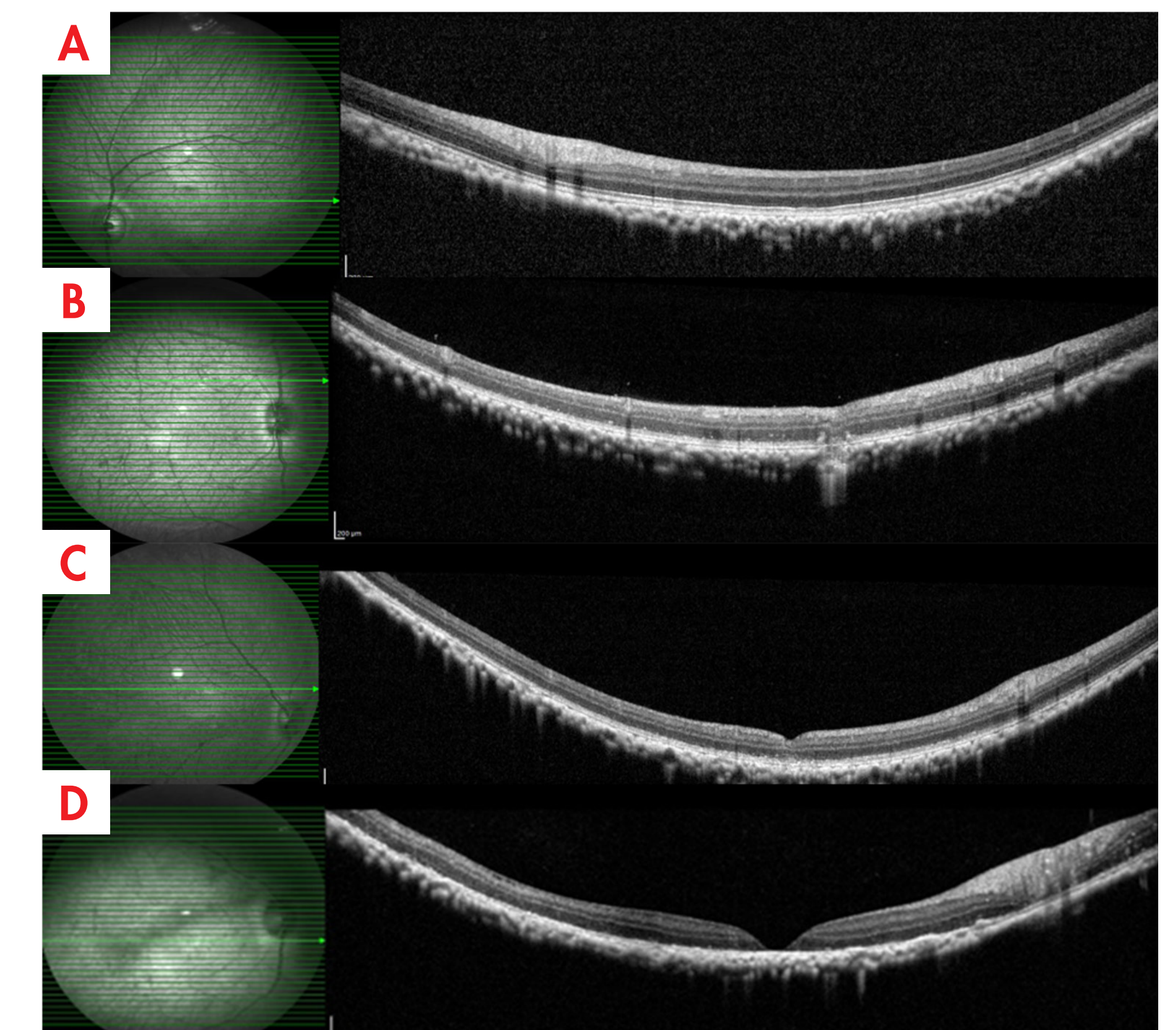


Figure 5. Obvious structural changes with dose-dependence were observed at the injection site by OCT in eyes dosed at different levels. OCT analysis was done at 13 weeks post dosing. (A. 1.5E10 vg/eye, B. 6.3E10 vg/eye, C.1.4E11 vg/eye, D. 2.4E12 vg/eye).

At the 2.4E12 vg/eye dose, no recovery of these morphological findings was observed at 13 weeks after injection. A tendency of aggravation in the dosed eyes was observed. This finding correlates with changes in retinal morphology, decreased amplitude or extended implicit time in the ERG.

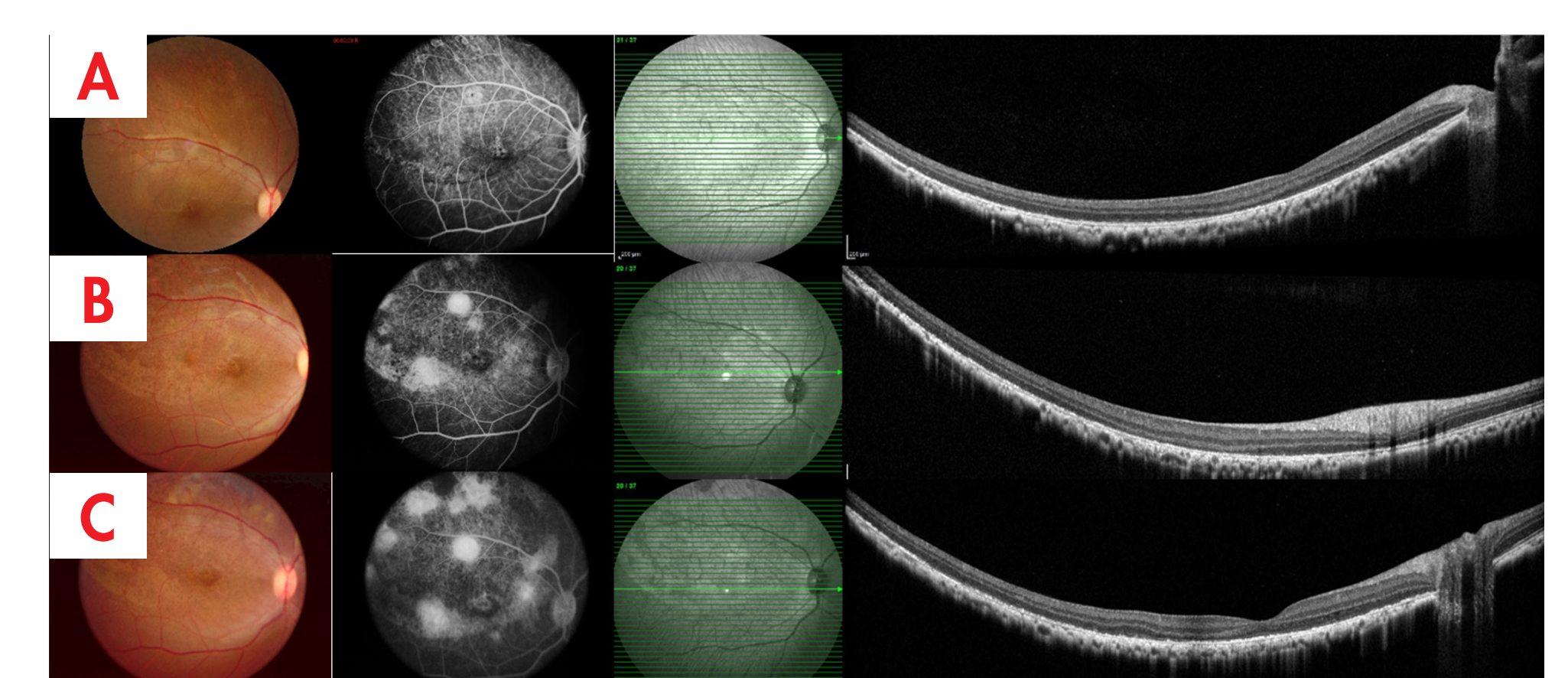


Figure 6. Multifocal hyper-fluorescence and structural changes of the outer retina aggravated over time at the dose level of 2.4E11 vg/eye in 4 weeks (A), 8 weeks (B) and 13 weeks (C) post dosing.

CONCLUSION

Subretinal injection could induce retinal pigment changes at the injection site. Ocular gene therapies at a dose level below 2.0E11 vg/eye were tolerated for retinal structure and function in cynomolgus monkeys. Higher doses may increase the risk of retinal toxicity, which needs to be fully considered in preclinical evaluations and clinical studies.