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Commentary
https://doi.org/10.63501/w407zz65

CRISPR-Corrected RPE Monolayer Patches for Geographic Atrophy in Age-Related Macular Degeneration: A Perspective

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ABSTRACT

Geographic atrophy (GA), the atrophic late stage of age-related macular degeneration (AMD), is a leading cause of irreversible central vision loss in older adults. While recent therapies may slow GA progression, broadly restorative interventions capable of replacing lost retinal tissue remain limited. Advances in tissue engineering have enabled polarized, scaffold-supported retinal pigment epithelium (RPE) monolayer patches that can be surgically implanted into GA lesions, with early clinical studies demonstrating feasibility, safety, and preliminary anatomical and functional signals. In parallel, CRISPR-based genome editing—including base and prime editing—has matured to allow increasingly precise correction or modulation of disease-associated alleles and pathways implicated in AMD, such as complement dysregulation, oxidative stress responses, lipid handling, and the ARMS2/HTRA1 locus. Integrating CRISPR correction into an autologous induced pluripotent stem cell (iPSC)–derived RPE patch strategy offers a dual-action therapeutic concept: (i) **structural replacement** of lost RPE with restoration of a polarized epithelial niche and (ii) **cell-intrinsic disease modification**, achieved by reducing genetic or pathway-level susceptibility to AMD pathology. This Perspective outlines the biological rationale, a pragmatic GMP-compatible manufacturing and quality-control framework, preclinical evaluation milestones, clinical endpoints, and key technical, safety, and regulatory guardrails required to advance CRISPR-corrected RPE monolayer patches toward first-in-human evaluation.

Keywords: *Retinal pigment epithelium; CRISPR; iPSC; geographic atrophy; age-related macular degeneration; tissue engineering; scaffold; cell therapy*

1. Clinical need and translational opportunity

GA is characterized by progressive loss of RPE, photoreceptors, and the choriocapillaris, often resulting in profound central vision impairment. Current AMD therapies largely target neovascular disease; GA remains without broadly effective restorative interventions, though some therapies may slow progression. RPE monolayer replacement addresses the immediate anatomic deficit by re-establishing a polarized epithelial interface, which is essential for photoreceptor support. Early clinical work with scaffold-supported RPE patches, both embryonic stem cell (hESC)-derived and engineered constructs, has demonstrated surgical feasibility, long-term survival, and preliminary signals of

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photoreceptor preservation and functional benefit (1–4,7). Uncorrected autologous cells may retain genetic and epigenetic susceptibilities that contributed to disease; therefore, CRISPR-corrected, autologous iPSC-RPE patches offer a combined structural and molecular remediation approach.

2. RPE & AMD biology: implications for design

RPE functions critical to retinal homeostasis include phagocytosis of photoreceptor outer segments, polarized secretion of trophic factors (VEGF, PEDF), antioxidative defenses, lipid handling, and immune regulation—particularly complement regulation. Genetic loci (CFH, C3, ARMS2/HTRA1) and polygenic risk influence these pathways, increasing RPE vulnerability to oxidative and immune-mediated injury. Correcting high-impact alleles or modulating pathway activity in autologous iPSCs may produce grafts with improved resilience in the GA microenvironment. Editing approaches should minimize the risk of double-strand breaks (DSBs) (favoring base/prime editors or high-fidelity nucleases) and target changes with demonstrable phenotypic benefit.

CRISPR Target Prioritization Framework

To balance feasibility, safety, and regulatory tractability:

- **Tier 1 (Most feasible):** Single-variant correction or focused pathway modulation with clear mechanistic linkage and measurable in-vitro rescue.
- **Tier 2 (Intermediate):** Multiplex edits targeting multiple pathway nodes, acknowledging regulatory and manufacturing complexity.
- **Tier 3 (Future direction):** Polygenic "risk optimization," longer-term approach, not first-in-human.

A first-in-human program would likely prioritize a single target with the clearest mechanistic linkage and measurable functional rescue in iPSC-derived RPE.

3. Materials and manufacturing strategy

3.1 Source cells & reprogramming

- Peripheral blood or skin fibroblasts were collected under informed consent.
- Integration-free reprogramming (Sendai virus, episomal vectors) to produce clinical-grade iPSCs under GMP.

3.2 Precision genome editing

- **Variant selection:** Prioritize pathogenic or high-effect AMD-associated alleles (CFH, functional ARMS2/HTRA1) or key pathway nodes.
- **Editing modality:** Base or prime editors preferred to avoid DSBs; high-fidelity Cas nucleases used when required.
- **Delivery and clonal selection:** Transient, non-integrating delivery (RNPs or mRNA) followed by clonal isolation, WGS-based off-target assessment, and selection of safe, correctly edited clones.

3.3 RPE differentiation and scaffold engineering

- Edited iPSC clones differentiated into mature, polarized RPE (pigmented, hexagonal morphology, competent phagocytosis).
- Seeded onto thin, transparent, biocompatible scaffold (parylene, ultrathin PLGA, or clinically validated membranes) optimized for surgical delivery and polarized secretion.

3.4 GMP QC and release testing

- **Genomic:** WGS to confirm on-target edit, absence of clinically relevant off-targets; karyotype and copy-number assessment.

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- **Residual pluripotency:** Absence of OCT4/NANOG; validated assays for undifferentiated cells.
- **Functional:** Phagocytosis assay, transepithelial resistance (TEER), VEGF/PEDF ratios, metabolic/oxidative challenge assays.
- **Sterility:** Endotoxin, mycoplasma, and adventitious agent testing.

All assays employ pre-specified, protocolized thresholds for GMP release.

4. Functional targets and measurable performance criteria

- **Anatomic survival & integration:** Continuous pigmented monolayer on OCT/FAF and histology in preclinical models.
- **Phagocytic competence:** Normalized outer segment uptake in functional assays.
- **Polarized secretion:** Physiologic VEGF: PEDF balance measured in vitro and locally.
- **Resilience to stressors:** Reduced complement activation and oxidative markers versus uncorrected controls.
- **Clinical function:** Photoreceptor layer preservation (OCT), microperimetry, reading speed/BCVA.

5. In-vitro and preclinical evaluation roadmap

In-vitro (release and mechanistic assays):

- Genomic safety: WGS, targeted off-target panels, transcriptome screens
- Functional RPE assays: Phagocytosis, TEER, polarized growth-factor secretion, response to oxidative/complement challenge
- Tumorigenicity proxies: Absence of pluripotency markers, soft-agar transformation assays

Small animals (rodents):

- Goals: Short-term engraftment, immune profiling, initial safety/tumorigenicity
- Endpoints: Histology, localized inflammation, biodistribution

Large animals (pig/NHP):

- Goals: Surgical feasibility, long-term survival, photoreceptor preservation, functional ERG, safety
- Endpoints: OCT, FAF, ERG, histology, systemic biodistribution, genomic safety assays

6. Design levers → biologic & translational criteria (Table 1)

Target	Rationale	Key Assays	Go / No-Go Criteria
Edit target & modality	Functional correction; minimize DSB risk	WGS, phenotype rescue	On-target confirmed; no deleterious rearrangements
iPSC clone selection	Prevent clonal risk	WGS, karyotype, CNV	No oncogenic variants; genomic stability
RPE differentiation	Functional maturity	TEER, pigmentation, phagocytosis	Pre-specified functional thresholds met
Scaffold design	Surgical & biological integration	Mechanical testing, secretion polarity	Integrity maintained; polarized secretion
Release testing	Patient safety	Sterility, pluripotency assays	All release criteria satisfied

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7. Preclinical endpoints assays and suggested group sizes (Table 2)

Endpoint	Assay	Suggested Scope
Genomic safety	WGS, off-target panels	3–5 clones
Short-term ocular safety	OCT, fundus, histology	8–10 rodents
Surgical feasibility	Implant retention & handling	6–8 large animals
Functional rescue	OCT, ERG, histology	6–10 per group
Tumorigenicity	Long-term histology	10–15 large animals

8. Regulatory and ethical considerations

Autologous cell therapy combined with genome editing engages ATMP and somatic genome editing frameworks. Considerations: comprehensive genomic safety datasets, validated GMP manufacturing, tumorigenicity testing, chain of identity, and informed consent. Early engagement with the FDA, EMA, and national regulators is essential. Ethical concerns: germline risk mitigation, incidental genomic findings, equitable patient selection.

Ethics statement: This article reports no new human or animal studies. Future work involving human cells or animals will require IRB/ethics approval and informed consent.

9. Clinical translation strategy

Phase I (first-in-human): Small safety-focused cohort of advanced GA patients.

- **Primary endpoints:** Ocular/systemic safety, graft survival (OCT/FAF), absence of ectopic proliferation/PVR
- **Secondary endpoints:** Photoreceptor rescue (OCT), microperimetry, BCVA, reading speed
- **Adaptive features:** Staged enrollment, genomic safety stopping rules, sentinel dosing
- **Manufacturing:** Autologous INDs initially slow; HLA-typed allogeneic banks with targeted correction may improve availability

10. Limitations and future directions

Challenges include the time and cost of autologous workflows, the need for exhaustive genomic safety, and the long-term durability in the GA microenvironment. Residual drivers (complement, drusen) may threaten graft longevity; local immunomodulation or complement inhibition may be required. Future directions: faster GMP editing, high-throughput off-target assays, immunomodulatory biomaterials, and scalable clinical translation.

CONCLUSION

CRISPR-corrected, scaffold-supported RPE monolayer patches pair structural tissue replacement with precision correction of cell-intrinsic disease drivers. Foundational RPE patchwork and advances in genome editing create a plausible translational path. Success requires rigorous genomic safety, reproducible GMP manufacturing, staged preclinical validation, and early regulatory engagement. If addressed methodically, this dual-modality approach could transform GA into a condition amenable to durable structural and molecular repair.

ABBREVIATIONS

- TEER: Transepithelial electrical resistance
- WGS: Whole-genome sequencing
- FAF: Fundus autofluorescence
- ERG: Electroretinography
- PVR: Proliferative vitreoretinopathy
- ATMP: Advanced therapy medicinal product

Conflict of Interest

None declared.

Ethical Statement

Not applicable. No new human/animal studies are reported, and future work will require IRB approval and informed consent.

AI Use Statement

Generative AI tools were used only for phrasing and grammar; all scientific concepts and conclusions are original.

Funding: None.

REFERENCES

1. da Cruz, L., Fynes, K., Georgiadis, O., Kerby, J., Luo, Y. H., Ahmado, A., Vernon, A., Daniels, J. T., Nommiste, B., Hasan, S. M., Gooljar, S. B., Carr, A. F., Vugler, A., Ramsden, C. M., Bictash, M., Fenster, M., Steer, J., Harbinson, T., Wilbrey, A., Tufail, A., … Coffey, P. J. (2018). Phase 1 clinical study of an embryonic stem cell-derived retinal pigment epithelium patch in age-related macular degeneration. *Nature biotechnology*, *36*(4), 328–337. https://doi.org/10.1038/nbt.4114
2. Mandai, M., Watanabe, A., Kurimoto, Y., Hirami, Y., Morinaga, C., Daimon, T., Fujihara, M., Akimaru, H., Sakai, N., Shibata, Y., Terada, M., Nomiya, Y., Tanishima, S., Nakamura, M., Kamao, H., Sugita, S., Onishi, A., Ito, T., Fujita, K., Kawamata, S., … Takahashi, M. (2017). Autologous Induced Stem-Cell-Derived Retinal Cells for Macular Degeneration. *The New England journal of medicine*, *376*(11), 1038–1046. https://doi.org/10.1056/NEJMoa1608368
3. Sharma, R., Khristov, V., Rising, A., Jha, B. S., Dejene, R., Hotaling, N., Li, Y., Stoddard, J., Stankewicz, C., Wan, Q., Zhang, C., Campos, M. M., Miyagishima, K. J., McGaughey, D., Villasmil, R., Mattapallil, M., Stanzel, B., Qian, H., Wong, W., Chase, L., … Bharti, K. (2019). Clinical-grade stem cell-derived retinal pigment epithelium patch rescues retinal degeneration in rodents and pigs. *Science translational medicine*, *11*(475), eaat5580. https://doi.org/10.1126/scitranslmed.aat5580
4. Kamao, H., Mandai, M., Ohashi, W., Hirami, Y., Kurimoto, Y., Kiryu, J., & Takahashi, M. (2017). Evaluation of the Surgical Device and Procedure for Extracellular Matrix-Scaffold-Supported Human iPSC-Derived Retinal Pigment Epithelium Cell Sheet Transplantation. *Investigative ophthalmology & visual science*, *58*(1), 211–220. https://doi.org/10.1167/iovs.16-19778
5. Mehat, M. S., Sundaram, V., Ripamonti, C., Robson, A. G., Smith, A. J., Borooah, S., Robinson, M., Rosenthal, A. N., Innes, W., Weleber, R. G., Lee, R. W. J., Crossland, M., Rubin, G. S., Dhillon, B., Steel, D. H. W., Anglade, E., Lanza, R. P., Ali, R. R., Michaelides, M., & Bainbridge, J. W. B. (2018). Transplantation of Human Embryonic Stem Cell-Derived Retinal Pigment Epithelial Cells in Macular Degeneration. *Ophthalmology*, *125*(11), 1765–1775. https://doi.org/10.1016/j.ophtha.2018.04.037
6. Bakondi, B., Lv, W., Lu, B., Jones, M. K., Tsai, Y., Kim, K. J., Levy, R., Akhtar, A. A., Breunig, J. J., Svendsen, C. N., & Wang, S. (2016). In Vivo CRISPR/Cas9 Gene Editing Corrects Retinal Dystrophy in the S334ter-3 Rat Model of Autosomal Dominant Retinitis Pigmentosa. *Molecular therapy : the journal of the American Society of Gene Therapy*, *24*(3), 556–563. https://doi.org/10.1038/mt.2015.220
7. Maeder ML, Stefanidakis M, Wilson CJ, et al. Development of a gene-editing approach to restore vision loss in Leber congenital amaurosis type 10. Nature Medicine. 2019 Feb;25(2):229-233. DOI: 10.1038/s41591-018-0327-9. PMID: 30664785
8. Tsai, S. Q., Zheng, Z., Nguyen, N. T., Liebers, M., Topkar, V. V., Thapar, V., Wyvekens, N., Khayter, C., Iafrate, A. J., Le, L. P., Aryee, M. J., & Joung, J. K. (2015). GUIDE-seq enables genome-wide profiling of off-target cleavage by CRISPR-Cas nucleases. *Nature biotechnology*, *33*(2), 187–197. https://doi.org/10.1038/nbt.3117
9. Hsu, P. D., Lander, E. S., & Zhang, F. (2014). Development and applications of CRISPR-Cas9 for genome engineering. *Cell*, *157*(6), 1262–1278. https://doi.org/10.1016/j.cell.2014.05.010

10. Kosicki, M., Tomberg, K., & Bradley, A. (2018). Repair of double-strand breaks induced by CRISPR-Cas9 leads to large deletions and complex rearrangements. *Nature biotechnology*, *36*(8), 765–771. https://doi.org/10.1038/nbt.4192