

General Comments

Wong and co-authors have produced an excellent study demonstrating the value of AS-OCT for producing predictive metrics for successful cell injection therapy. This study is of significant interest to the field. This is especially the case given the exceptional experimental and statistical rigor. The basis of their hypothesis and the approaches used are scientifically sound. This study will form the basis for how others can assess and predict corneal endothelial therapy success, which would be a primary concern of patients undergoing the procedure. Very excited to see how this study positively impacts the field, and to see this approach translated to primate models and ultimately into humans. Nevertheless, prior to publication, I ask the authors to address the points and concerns I express in this review.

General

1. Citation of relevant research is limited, and absence of specific studies is conspicuous. I strongly encourage the authors, who know the field extremely well, to include additional citations to highly relevant research. I provide some examples below but ask the authors to reassess their citations throughout. If citation is limited by journal guidance, I encourage the authors to submit a supplemental reference list.
2. This study demonstrated that the SNECi were significantly better in the early time points, but not at the last time point investigated. Measurement well beyond (months) the time points assessed in this study would have been of significant interest. What is the reason that the authors used a relatively short time range to study success? This is especially important because one of the observations made by the Kinoshita group in their 5 year follow up was marked and consistent cell loss (stabilizing at about year 4-5). Is the mouse model not suitable for assessing long-term cell loss? (Kohsaku et al., Ophthalmology 2021).

Comments

Introduction, Page 2, line 74. The literature has several other studies in addition to the authors previous study (citation 15) where viable protocols were developed, the authors are encouraged to cite them (e.g., Okomura et al., Cornea 2014; Bartakova, et al., IOVS 2018; Santerre et al., Methods in Molecular Biology 2020).

Introduction, Page 2, line 92. The authors are encouraged to include a brief explanation for the basis of their hypothesis (aggregates as a predictor of deturgescence). Alluding to it for the first time in the Discussion section is not sufficient (lines 314-16).

Materials and Methods, Page 4, line 177. The number of times the cells were passaged before use for injections must be stated in the "Corneal Endotheli Cell Injections" subsection. It is insufficient to ask the reader to refer to a previous study. This is of critical importance since there is at least one significant outcome consideration depending on the number of passages (I point one out later in this review - senescence).

Results, Page 5, lines 233, 234 and 236. The term “case” here from a medical publishing standpoint is unclear. I prefer to restrict its use to human subjects. Please consider other more clear terms. In this case I recommend changing each instance to “deturgescence” or “animals” or “eyes” as appropriate.

Results, Page 5, line 241. Please change “case” here to “animals” or “eyes.”

Results, Page 6, line 259. Please change “case” here to “animals” or “eyes.”

Results, Page 8, line 275-78. Please change “case” here to “animals” or “eyes.”

Results, Figure 1, page 8. This figure could benefit from some reformatting. In its current state it leaves a desire for greater aesthetic that can help the reader more quickly assess the results. Day/baseline labels should be removed, and a single label should be added to the top of each column. In addition, the animal number (e.g., “J92”) should be removed from the image and placed to the left of each row. A white line/gap between rows would be helpful.

Discussion, Page 9, line 284. The authors should address key differences between the cultures produced in the Kinoshita et al., NEJM 2018 and this study. The authors should briefly discuss if differences in culture method may contribute to differences results obtained with AS-OCT.

Discussion, Page 9, line 284. In addition to addressing the differences between the culture methods, the authors also must address a key limitation in the cell injection approach. Not all the cells that are injected adhere to the posterior side of the cornea. In fact, in the Kinoshita et al., 2018 study, the investigators injected 1 million cells. At best, about 1/5th of those cells initially adhered. What happened to the other 750,000 cells? Surely, some underwent cell death (programmed cell death perhaps), some may have found their way to other locations within the anterior chamber or (as unlikely as it is believed to be) found their way to extraocular tissues. The Okumura, et al., Sci. Reports 2016 study where the Kinoshita group attempted to address this is insufficient, namely because the assay they used to measure the presence of human DNA in extraocular tissue is not sensitive enough. A long-term study is needed, and a decades-level follow up of human subjects will ultimately be needed.

Because expansion of corneal endothelial cells in culture makes the most economical and clinical sense (i.e, addressing the worldwide shortage), cellular senesce will remain a concern (Okumura et al., Sci. Reports 2015; Hongo et al., IOVS 2017, Frausto et al, Sci. Reports, 2020). While pre-senescent or cells in the early stages of senescence may function well if they adhere to the cornea, any that do find themselves in other tissues have the potential (in theory) to induce cancer in adjacent tissue (Lau and David, Expert Opinion on Therapeutic Targets 2019; Georgilis et al., Cancer Cell 2018). To get sufficient cells for injections. **As such, authors should point out that while the cell injection method remains a leading option for corneal endothelial-based therapy (especially in older individuals), other approaches are being investigated that are poised to remove the primary long-term concern with cell injection therapy.** So long as I continue to review studies using the cell injection method, and until that time that a sufficiently sensitive method is used to detect DNA in extraocular tissues (or a decades long study in humans/primates), I will continue to raise this concern,

especially considering an alternative method exists (i.e., biomimetic synthesized Descemet membrane derivatives for use in DMEK) and remains a continued area of research. This approach will be less wasteful (may be able to treat 2-3x more individuals compared with cell injection), and would likely remove any concern that senescent cells would have on ocular or extraocular tissue.

Discussion, Page 10, line 324. For clarity, please change “case” to “animal” or “eye.”

Discussion, Page 11, lines 346-345. In my reading of the manuscript, there was nothing to suggest that studying the differences between CEC and SNECi is particularly warranted, unless to improve the CEC approach. The authors should clarify that, at least as I understand the field, there is no expectation that SNECi would ever become clinically viable given the many surgical techniques available, therefore necessitating CEC expansion. Apology if I misunderstood, but some clarity here would be helpful.

Limitations

1. The authors do a wonderful job addressing various limitations. However, I did not see them qualify their study with the acknowledgement that this is in a rabbit model. While an excellent animal model, they're not human (or primate) and the authors should address why the results remain valid.
2. I am cognizant that this study is focused on the utility of AS-OCT as a tool to predict cell injection therapy success, but we must remain sober to the potential long-term effects of cell injection therapy. Until that time where those concerns are sufficiently and scientifically addressed, a very brief recognition, in the form of a “limitation,” that senescence and its potential long-term effect is warranted.