Author Response: Tear Levels of 8-hydroxy-2′-deoxyguanosine

The authors would like to thank Tong\textsuperscript{1} for pointing out the potential influence of nuclease activity on extracellular DNA (eDNA) in human tears on measurable quantities of 8-hydroxy-2′-deoxyguanosine (8OHDG). Arguably, the majority of DNA components measured in tears may be expected to be extracellular given the tears are fluid rather than tissue based.

Work reporting decreased nuclease activity as it relates to dry eye supports increased eDNA as a potential influence on quantification of eDNA in tears.\textsuperscript{2,3} However, small sample sizes reported to date preclude firm scientific conclusions regarding significance. Method of sample collection will influence outcomes; Schirmer strips to collect tear samples as reported by Sonawane\textsuperscript{2} and conjunctival washings prior to tear collection reported by Tibrewal\textsuperscript{3} have greater potential for collection of cells from the conjunctival and/or skin surface than the use of glass microcapillary tubes without washings as used in our study.\textsuperscript{4} We acknowledge collection of conjunctival cells through impression membranes is a viable method to explore direct cellular 8OHDG evaluation on the ocular surface. However, much work remains to determine significance when comparing 8OHDG expression directly from cellular samples with 8OHDG in human tears. Subject discomfort and variability of cell collection from impression cytology may also confound results. Hence, the noninvasive nature of tear collection by glass microcapillary tube may ultimately prove a useful parameter in assessment of ocular surface 8OHDG expression. To our knowledge, 8OHDG expression of tears and conjunctival cell sample types have not been adequately compared. Furthermore, as stated in our manuscript, we acknowledge dry eye as a potential confounder in our study and recommend additional studies in various clinical populations, such as those with dry eye, are important to establish the significance of our findings.\textsuperscript{5} Ultimately, it may be shown 8OHDG expression in tears (primarily eDNA) compared with conjunctival cells (combined cellular and eDNA) will assist with furthering accurate subclassification of those who suffer ocular surface disease.

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