Absorbance Imaging and Artificial Intelligence for Assessing Quality of Manufactured Retinal Pigment Epithelium

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Statement of Purpose: In tissue engineering applications, the light microscope is frequently used to non-invasively assess quality during manufacturing [1]. Our goal was to make these analyses more quantitative. For induced pluripotent stem cell-derived retinal pigment epithelium (iPSC-RPE), this was achieved through absorbance imaging and artificial intelligence algorithms.

Methods: Clinical-grade iPSCs were generated from CD34+ monocytes isolated from the peripheral blood mononuclear cell fraction of blood from healthy donors using 4 episomal factors. iPSCs were used to generate RPE (2) that were matured in culture under current good manufacturing practice conditions (cGMP). RPE were matured for 8 weeks in 12-well transwell plates under default conditions (Control), with HPI4 to inhibit maturation (HPI4) and with aphidicolin to enhance maturation (Aphidicolin). Once per week, RPE quality was assessed by measuring transepithelial resistance (TER) using "chopstick-style" electrodes. RPE were also imaged by absorbance imaging, which is a quantitative imaging routine that can be performed on any brightfield microscope. RPE expression of melanin increases as they mature causing RPE to absorb light. Brightfield image captures of the RPE (I) are "divided" by image captures of background (I_{max}, transwell plate without cells) using this relationship: $Abs = -\log_{10}(I/I_{max})$. In this way, each sensor in the camera array serves as a tiny spectrophotometer that generates a quantitative measurement of the amount of light absorbed by the RPE. This ratiometric imaging approach enhances data comparability, which will be important during scale-up where consistency over thousands of manufactured units is desired. Using the images and TER data, a deep neural network (DNN) was trained to predict TER from images. The DNN was tested by asking it to predict the TER from images that were not used in training.

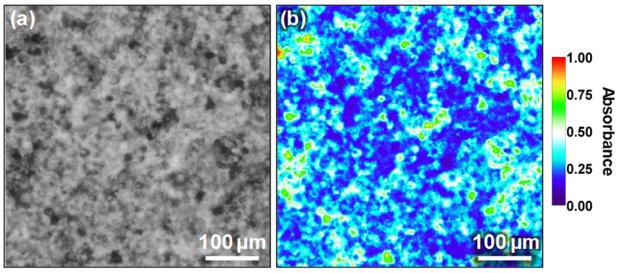

Fig. 1. (a) Brightfield & (b) absorbance images of Control RPE from the same field of view. *Right:* Absorbance scale applies to (b).

Results: The ability to measure absorbance with a bightfield microscope plus camera was verified using a range of neutral density (ND) filters and yeilded a linear response ($R^2 = 0.999$). The comparability of absorbance values across three microscope-camera systems was assessed with the ND filters yielding a root mean square error (RMSE) of 0.07 absorbance units between systems. A brightfield image and absorbance image of RPE are shown in Fig. 1. The mean absorbance of a well of RPE correlated weakly with the measured TER (Fig. 1a) ($R^2 = 0.19$). In contrast, when a trained DNN was shown images from wells of RPE that were not used for training, the TER that the DNN predicted strongly correlated with the measured TER (Fig. 2b) ($R^2 = 0.97$). Of the 36 wells of RPE analyzed in Fig. 2b, only 1 false negative and 0 false positives were detected.

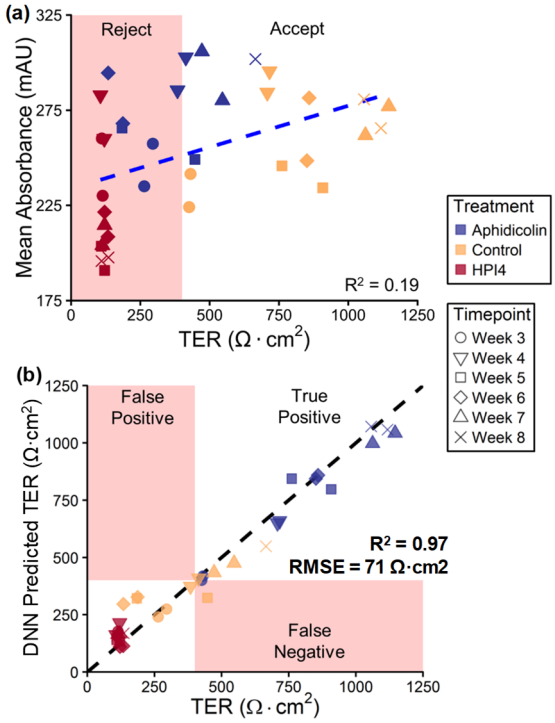

Fig. 2. (a) Measured TER plotted against mean absorbance from each well of RPE. (b) The trained DNN was asked to predict the measured TER from images that it had not seen during training. (a,b) Each data point in the plots was derived from one well in a 12-well transwell plate. The same wells were analyzed for both plots and the x-axis is the TER that was measured by the electrodes for each well. The dotted lines in each plot represent a perfect prediction. A "quality threshold" of 400 Ω·cm2 was assigned as a release criteria (as deteremined from historical data) and used to determine false positives and false negatives.

Conclusions: An absorbance imaging method was developed for quantitative, comparable, non-invasive, imaging of RPE that may be suitable for manufacturing scale-up. A DNN was able to predict RPE function (measured TER) from absorbance images, providing a proof of concept that tissue function can be predicted from non-invasive image captures.

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