Subpopulation Identification During Cell-Material Interactions Via Neural Network Clustering of Motility Metrics

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Statement of Purpose: *In vitro* biomaterial microenvironments are used as platforms to enable research in many areas in which identification of subpopulations within heterogeneous cell populations are of great importance, including cancer cell biology, developmental biology, and mechanobiology[1,2,3]. Moreover, the recent emphasis on designing programmable and dynamic biomaterial microenvironments has added to the need for methods of identifying cell subpopulations in both space and *time*[4,5,6]. To ensure that biomaterial platforms continue to drive advances in these and other areas, the goal of the present study was to develop a non-destructive, non-invasive, microscopy-based method for identifying cell subpopulations that demonstrate distinct motility behavior when interacting with each other and with biomaterial microenvironments. The approach was to perform neural network clustering of motility metrics derived from automated cell tracking data.

Methods: Neural network clustering was applied to motility metrics output from a recently developed automated cell-tracking algorithm, termed ACT*IV*E (Automated Contour-based Tracking for *In Vitro* Environments)[7]. The MATLAB "Neural Network Clustering" module was used to implement neural-network-based clustering and identification of cell subpopulations. To test the approach, the method was applied to a dataset in which motility metrics from two distinct populations were combined to produce a single, heterogeneous population in which every cell was derived from one of two known populations. To generate the two populations, ACT*IV*E was used to analyze cell motility on two substrates: a substrate comprising aligned sub-micron scale wrinkles and flat tissue culture polystyrene (TCPS)[8]. On both substrates, C3H10T1/2 cells were seeded at 20,000 cells per cm^2, stained with Hoechst dye, and imaged for 24 hours. The neural network was trained using the following motility metrics from ACT*IV*E: 1) average major cell axis, 2) average minor cell axis, 3) average cell area, 4) average velocity in the x direction, 5) average velocity in the y direction, 6) average large eigenvalue component of the gyration tensor, 7) average small eigenvalue component of the gyration tensor, and 8) average asphericity.

Results: Following training of the neural network, reclassification of the cell data within the network revealed two distinct cell subpopulations corresponding to the cells that had been cultured on the two different substrate topographies. Heat maps, which display the trained weights for each individual cell feature (Figure 1) indicate that the two cell populations are predominantly influenced by the following metrics in no particular order: average major cell axis (1), average minor cell axis (2), average cell area (3), average large eigenvalue component of the gyration tensor (6), and average small eigenvalue component of the gyration tensor (7). The relative importance of these particular metrics suggests distinct nuclear morphology and track shape between the two substrate environments.

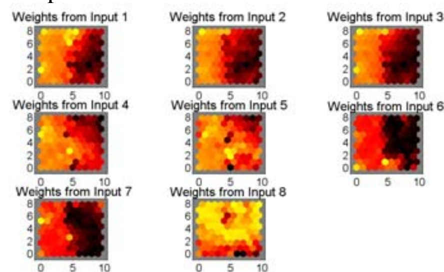

Figure 1: Heat map displaying clustering characteristics for each cell feature. Values for each feature were rescaled to reflect a [-1,1] range from black to red, respectively. Input numbers as defined in the text.

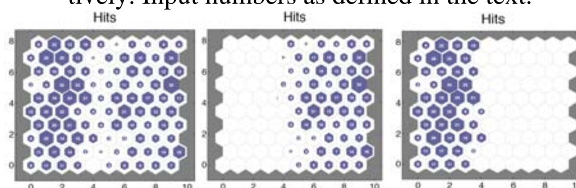

Figure 2: The identification of subpopulations within the heterogeneous population. A) Hit map where each cell is associated with its best matching node within the network. By isolating the B) wrinkled and C) TCPS data, clustering of distinct subpopulations is confirmed.

Each cell was matched to its corresponding node in the network most closely associated with its scaled features (Figure 2A). The resulting clusters (left/right clusters apparent in Figure 2A) were validated as distinct subpopulations by producing independent hit maps for the wrinkled and TCPS data sets (Figure 2B,C). The information from the figures above indicates that this approach is capable not only of identifying cell subpopulations but also of determining quantitative cell motility characteristics that define the subpopulations.

Conclusions: The results suggest that neural network clustering of motility metrics derived from automated cell tracking may provide a non-destructive, non-invasive, microscopy-based method for identifying cell subpopulations. When applied in combination with biomaterial microenvironments that are available or in development, this approach is anticipated to enable new strategies for identifying and studying cell subpopulations relevant to cancer, tissue development, and regenerative medicine.

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