Supplemental Information

Area-based Image Analysis Algorithm for Quantification of Macrophage–fibroblast Cocultures

Morphological differences in image analysis of macrophage and fibroblast cocultures

We used RAW 264.7 murine BALB/c-derived monocyte/macrophage cells that typically present an adherent, rounded morphology when viable. Most viable cells are loosely attached, with greater roundedness observed for dense populations. It is not uncommon to see RAW 264.7 macrophages present spindle or stellate morphologies when activated with cytokines towards their inflammatory or anti-inflammatory phenotypes[1]. NIH 3T3 cells are derived from NIH/Swiss mice. This fibroblast cell line presents large, flat, and often spindle-shaped morphologies. Upon activation to their proto or myofibroblast forms, they show clear spindle formations with tight actin bundles at their extreme peripheries[2]. Morphometric parameters such as elongation factor aspect ratio have been used previously to distinguish macrophages in their naïve versus polarized phenotypes[3,4]. Here, the elongation factor, calculated as the ratio of the greatest length of the cell divided by the shortest perpendicular distance bisecting the nucleus, would provide a quantitative metric with potential utility within this algorithm for further increasing the accuracy of distinguishing macrophages versus fibroblasts (or other cell types). For any coculture, optimizing this elongation factor-based approach for cell identification would require the statistical identification of an appropriate elongation factor cut-off value that would reliably signify, for example, fibroblasts versus macrophages within an autodetection algorithm. With certain cell types having activated states that produce significant variations in cell area[5], such morphometric analyses might also be adapted to provide further information regarding cellular activity/phenotype. These morphometric identification processes would be incorporated within the initial steps of the algorithm, prior to cell quantification calculations.

References:

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