

***In Situ* Quantification of Subpopulation Behavior in Heterogeneous Cell**

Populations: Implications for Tissue Regeneration

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INTRODUCTION: Advances in quantitative molecular- and cellular-biology highlight the need for correspondingly quantitative tissue-biology. We present a novel, widely applicable and non-invasive means to quantify behavior of subpopulations in mixed-cell cultures, using adhesion patterns within mesenchymal populations as an illustration. Relationships between the underlying statistical distributions of cell areas on various surfaces are considered using a combination of graphical and nonparametric statistical analysis and it is shown that activity can be accurately quantified in tissue subpopulations smaller than 1% by cell number using this method. Presence and behavior of a number of specific mesenchymal subpopulations are outlined, and implications for the translation from cell- to tissue-biology are discussed.

METHODS: We seeded fluorescently labelled STRO-1 selected and unselected and MG63 samples on tissue culture plastic and fibronectin coated surfaces. Cells were then fixed and randomly photographed. A large number of individual cell areas were then obtained per sample using retrospective image analysis. The distributions of cell areas for each cell type and surface were investigated by calculating percentile points, which provide a simple way to elucidate structure. We then constructed Percentile-Percentile (P-P) plots, which constitute a sensitive non-parametric means to compare relationships between underlying statistical distributions in different sets of data. In particular, if two datasets have distributions which are significantly different then the P-P plot will be nonlinear with the degree of nonlinearity relating directly to the degree of distributional disparity between the two datasets. Changes in the distribution of cell areas upon surface modification were used to determine the presence and activity of subpopulations with varying responses to fibronectin. Differences in the shapes of the distributions of cell areas from different mesenchymal fractions were used to

further elucidate differences in composition between them.

RESULTS: We detected the presence and activity of significant subpopulations in both STRO-1 selected and unselected mesenchymal fractions. Crucially, we identified the presence and activity of morphologically indistinct subpopulations. The continuous cell line MG63 was used as a negative control in which no subpopulations were present or active.

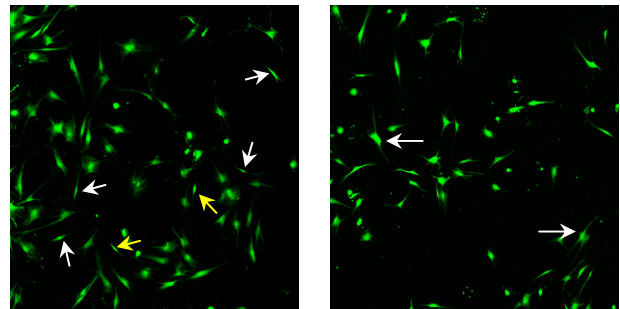


Fig. 1: Significant, morphologically distinct subpopulations are identified in both STRO-1 selected (right) and unselected (left) fractions. Yellow arrows highlight a morphologically indistinct subset of the white arrowed STRO-1⁺ subpopulation which behave in a significantly different manner.

DISCUSSION & CONCLUSIONS: We present a simple method which uses fluorescence labeling in combination with image analysis and mathematical data processing to detect the presence and activity of cell subpopulations retrospectively, in a non-invasive manner. Although we consider cell area in response to surface modification, recent advances in fluorescence cell- and protein-labeling mean that this method can be used to characterize behavior using a wide variety of biochemical-, protein- or alternative phenotypic-markers within any mixed cell population. This has significant implications to advance understanding of *in situ* tissue regeneration.