Assessing Preferred Proximity between Different Types of Embryonic Stem Cells

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Abstract: Embryonic stem cells (ESCs) studies play an important role for understanding the molecular events that underlie cell lineage commitment and serve as models for the development of disease. However, the interactions between neighboring embryonic stem cells are not fully understood. Assessing proximity between different types of embryonic stem cells might provide more information about distinct behaviors of embryonic stem cells. In this study, we processed 186 cell colonies on disc constrained microdomains and 152 cell colonies on ellipse. We grouped cell colonies based on different observed patterns and grouped cells by their locations. By applying two measurements on embryonic stem cell colonies, minimum spanning tree and average distance to the five closest objects, we investigated the difference of proximity between different types of embryonic stem cells, the difference between grouped cell colonies and the difference between grouped cells. We found one type of ESC has a smaller average path based on minimum spanning tree and higher proximity than the other type. We report consistent results for different types of embryonic stem cells: these findings may be useful to set benchmarks for empirical models which replicate ESC behaviors.

1 INTRODUCTION

Embryonic stem cells (ESCs) have shown potential for regenerative medicine towards the development of novel therapies for a wide range of diseases or injuries (Wu and Hochedlinger, 2011). ESCs can self-renew and differentiate into almost any type of mature cells. Due to the special properties of ESCs, they are useful for: 1) understanding the molecular events that regulate stemness and cell lineage commitment (Ivey et al., 2008)(Gan et al., 2007); 2) modeling development of disease (Avior et al., 2016).

Previous work has demonstrated that stem cells have the ability to socialize with their neighbors while interacting with their micro-environment (Fuchs et al., 2004). Recent studies illustrate that different types of ESCs have distinct dynamic social behaviors, such as variable migration speed and preferred number of neighbors (Phadnis et al., 2015). However, the neighboring effects in ESCs are still not fully understood as the underlying mechanisms of their communication are highly complex (Madl and Heilshorn, 2018)(Watt and Hogan, 2000). Understanding neighboring effects can benefit the investigation of behaviors in ESCs, which would be helpful for controlling differentiation and contribute towards the development of new stem cell therapies.

Brachyury (T) is a marker of early mesendodermal expression (Beddington et al., 1992)(Wilkinson et al., 1990). T+ cells are early differentiated cells as they are marked by Brachyury (T), while T- cells are naive ESCs. The process of spatial pattern formation in differentiated stem cells is essential for establishing functional mammalian tissue. Blin and colleagues demonstrated that T+ and T- cells prefer different numbers of neighboring cells, with T- cells preferring more neighbors than T+ cells (Blin et al., 2018).

In this study we extend on these findings and look into the preferred proximity (closeness) of neighbors between the two types of cells. We applied two new measurements to quantify the proximity of T+ and T- cells in ESC colonies and assess the difference of proximity within different patterning constraints.

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2 METHODS

2.1 Empirical Data

We used previous data of ESC colonies consisting of 186 images for disc experiments and 152 images for ellipse experiments (Blin et al., 2018)(Wang et al., 2018). Each image corresponds to a single cell colony that was captured 48 hours after the initial seeding of the stem cells. Each colony was randomly seeded with both T+ and T- cells. Populations of each cell type were isolated in the images by thresholding labelled image data. We extracted the cells' locations (described by x and y values) based on the centers of the nuclei. For disc experiments, we selected cells within 95.5 μm from the center of the disc; for ellipse experiments, we selected cells within the ellipse with the semi-major axis as 193.5 μm and semi-minor axis as 47 μm. Cells outside these defined constrained areas were considered as random noise and discarded. Figure 1 shows the indicative plots for disc and ellipse experiments.

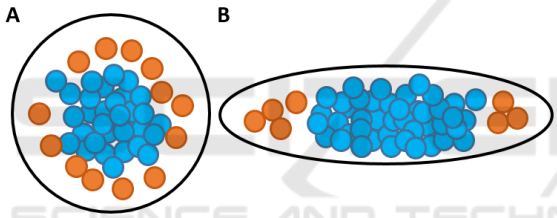

Figure 1: Indicative plots for A) disc and B) ellipse experiments. Blue circles represent T- cells; orange circles represent T+ cells.

2.2 Pattern Grouping

For both disc and ellipse experiments, we applied 2D kernel density estimation (Botev et al., 2010) to obtain the density maps of aggregated T- and T+ cells separately. Since we are interested in the locations of T+ cells, we thresholded the aggregated density map of T+ cells based on the mean of max and min density of each pixel to obtain the areas with high density T+ cells. The borders of high density areas (HDA) were smoothed by least squared circle or ellipse function. Figure 2 shows indicative plots for HDA on the disc and ellipse constrained microdomain. In the disc experiments, the HDA is a ring shaped area due to the symmetry of the disc. In the ellipse, the HDA is focused at the tips of the major axis of the ellipse. We compared the ratio of HDA/non-HDA size to the ratio of HDA/non-HDA cell number to get an indication of cell spacing. Subsequently we obtained three distinct groups of different cell patterns based on the grouping of T+ cells: 1) cell colonies with relatively higher density of T+ cells on HDA; 2) cell colonies with relatively low density of T+ cells on HDA; 3) cell colonies with no T+ cells.

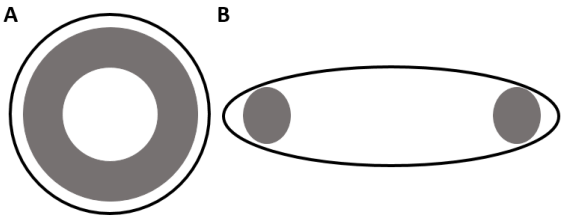

Figure 2: Indicative plots for HDA on A) disc and B) ellipse experiments.

2.3 Measurements of Neighboring Effects

2.3.1 Minimum Spanning Tree

We built a connected graph (Wilson, 1996) for T+ or T- cells in each cell colony by taking each cell as a node and connecting any two nodes with an edge. The weight of each edge was defined as the Euclidean distance between the two nodes. A spanning tree is a sub-graph that connects every node in the graph without any cycles (Kruskal, 1956). As there are different spanning trees for the graph, the minimum spanning tree is the spanning tree connecting the nodes through edges and has the smallest total weight (Prim, 1957). Therefore, the minimum spanning tree calculates the shortest path that connects all T+ or T- cells within the colony. We calculated the average path distance between two nodes (cells) by dividing the path distance of the minimum spanning tree by the number of T+ or T- cells respectively. A smaller average path distance indicates a higher proximity. Following this, we applied kernel density estimation (Botev et al., 2010) of the average path distance we received from T+ and T- cells from each cell colony. The number of mesh points used in the kernel density estimation was 256. We also calculated the minimum spanning tree for T+ and T- cells in each pattern group.

2.3.2 Quantifying Average Distance of Each Query Object to Five Nearest Targets

For each T+ or T- cell (i.e., object) within disc and ellipse experiments, we found the five closest T+ and T- cells (i.e., targets). We calculated the average distance from each object to its targets (referred to as D). The data presented four different cases of calculating D: 1) the object is a T- cell with T- cell targets; 2) the object is a T- cell with T+ cell targets; 3) the object

is a T+ cell with T- cell targets; and 4) the object is a T+ cell with T+ cell targets. For these four cases, we applied kernel density estimation (Botev et al., 2010) of D from all cells within each pattern group. Based on the borders of the HDA, we applied kernel density estimation of the cells in the HDA and cells outside the HDA separately to investigate the difference in proximity between the different cell type with both regions.

3 RESULTS

3.1 Pattern Groups

Figure 3 shows the percentage of different patterns observed in the disc and ellipse experiments. We defined pattern 1 as a relatively higher density of T+ cells within the HDA. In pattern 2, the density of T+ cells within the HDA was lower than the density of T+ cells within the remaining areas. On pattern 3, there were no T+ cells.

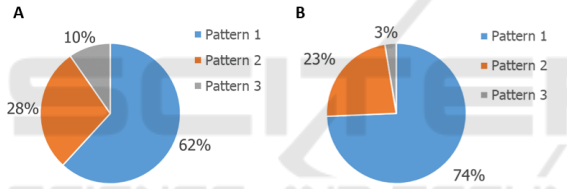

Figure 3: The percentage of the 3 different patterns observed within the A) disc and B) ellipse experiments. Pattern 1: high density of T+ cells within the HDA. Pattern 2: density of T+ cells within the HDA was lower than the density of T+ cells within the remaining areas. Pattern 3: no T+ cells.

3.2 Minimum Spanning Tree Results

The results of the overall distribution of average path distance based on the minimum spanning tree are shown in Figure 4. The overall aggregate results for the disc and the ellipse were consistent. It is noteworthy that T- cells have much smaller average path distance than T+ cells. The results indicate that T- cells have a higher proximity than T+ cells, and the variation in proximity of T+ cells was greater than in T- cells.

Figure 5 shows the kernel density estimation results from the pattern groups described in 3.1. The difference found in T- cells between the pattern groups was relatively small. For Pattern 3, in which there are no T+ cells, T- cells have the highest proximity. T+ cells in Pattern 1 (more T+ cells on HDA) are slightly more compact than T+ cells in Pattern 2. Again, the results from disc and ellipse experiments are consistent. The double peaks of T+ cells on ellipse experiments might due to the fact that in some colonies T+ cells were more dense at one tip of the ellipse and in some colonies T+ cells were more dense at both tips.

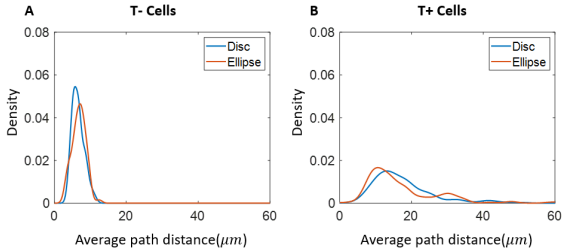

Figure 4: Kernel density estimation of average path distance (μm) of A) T- cells and B) T+ cells within disc and ellipse experiments.

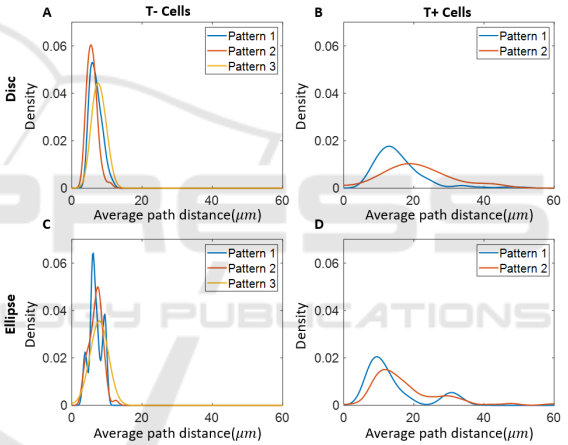

Figure 5: Kernel density estimation of average path distance of A) T- cells on disc experiments; B) T+ cells on disc experiments; C) T- cells on ellipse experiments; D) T+ cells on ellipse experiments.

3.3 Results of Average Distance of Each Object to Five Nearest Targets

Figure 6 shows the kernel density estimation results of average distance from the object to the five closest targets (D) from the aggregated cells on disc and ellipse experiments. The case of T- as the object and T- cells as the target results in the highest peak density in both disc and ellipse experiments. This is consistent with the results from the minimum spanning tree which showed that T- cells have a high proximity. For the case in which T- cells are taken as objects and T+ cells as targets the distribution of D has a high mean value and a wide spread. This result could be due to

the fact that there are more T- cells than T+ cells on the colonies. Hence, for T- cells it takes longer distance to retrieve for the closest T+ cells in average. For T+ cells, the distribution of D to its five closest T+ or T- cells are relatively similar.

Figure 7 shows the kernel density estimation results of D which is grouped by cell locations. It shows for all object T- cells the average D of the five closest T- cells showed relatively low variety whether contained within the HDA or not. For T- cells not in the HDA, the average distance to the five closest T+ cells is higher than for T- cells in the HDA. Interestingly, for the cases in which T+ cells are taken as objects, and both T- or T+ cells as targets, the T+ cells in the HDA have a higher average D than T+ cells not in the HDA and the distribution of D is slightly more spread out.

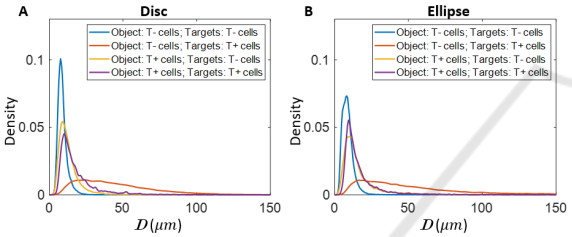

Figure 6: Kernel density estimation results of D from the aggregated cells on A) disc and B) ellipse experiments.

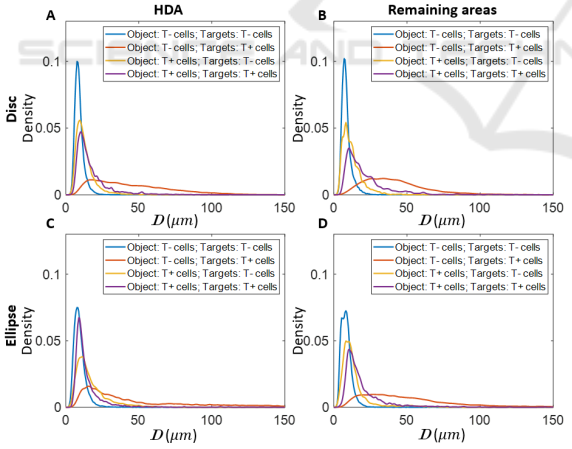

Figure 7: Kernel density estimation results of D from the aggregated A) object cells on HDA on disc; B) object cells on remaining areas on disc; C) object cells on HDA on ellipse; D) object cells on remaining areas on ellipse.

4 DISCUSSION

In this study, we quantified the proximity of different types of cells in ESC colonies on different shaped microdomains. We assessed cells' proximity in different patterns groups, and the difference of the proximity between different types of cells depending on location (as shown in Figure 5 and Figure 7). The obtained results are consistent across discs and ellipses, which provides some confidence on the generalizability of the extracted concepts (as shown in Figure 4 and Figure 6).

The results of the average path distance based minimum spanning tree indicate that T- cells prefer a higher proximity than T+ cells. The T+ cells in colonies that formed the pattern with more cells in the HDA (Pattern 1) have a higher proximity than the T+ cells in the other pattern groups. The results from D are consistent with the results from the minimum spanning tree that T- cells have a higher proximity than T+ cells. It also suggests that T+ cells have a tendency to stay away from other cells as they have a relatively low proximity to both T- and other T+ cells, compared to the high proximity we observed in T- cells.

Compared to our previous work, we have provided a more thorough analysis of the distribution of different measurements by applying kernel density estimation. We also analysed the difference between different observed patterns and different types of cells. This information further improves our understanding of the difference between types of stem cells, which could be useful for controlling the differentiation in ESCs. To the best of our knowledge, we are not aware of other studies in literature which have investigated the proximity in embryonic stem cells with the marker Brachyury (T).

Currently, we are building diverse ESC computational models to reproduce the pattern formation by investigating parsimonious, minimal sets of different social behaviors of different types of ESCs which replicate experimental data. This study is a step towards informing the hypothesis that we can test in our models as T+ cells tend to stay away from other cells.

In conclusion, we provided quantitative information regarding the proximities of T- and T+ cells. T+ cells have a tendency to stay away from other cells according to the results from the minimum spanning tree and D. This finding could be useful in the analysis of different social behaviors between different types of ESCs.

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