(Form 1)

Kyoto University	Doctor of Philosophy in Life Sciences	Name	LUO, Shuangyu
Thesis Title	Quantitative analysis of coordinated epithelial rotation on a two-dimensional discoidal pattern		

(Thesis Summary)

Among a number of dynamic cell behaviors during animal life, collective cell migration covers a variety of coordinated movements that are exhibited by tightly interconnected cells. One unique type of such collective cell migration in vivo is coordinated epithelial rotation in development, which has been modeled in vitro under spatially fine-tuned conditions. Despite the well-established role of cell-cell interactions in such coordinated rotational motion, the contribution of E-cadherin, a major homophilic cell-cell adhesion molecule in epithelia, has not been directly addressed on two-dimensional (2D) confined environments.

Benefiting from the microcontact printing technique, the applicant used MDCK (Madin-Darby canine kidney) II cells, one of the popular model mammalian epithelial cell lines, generated 2D monolayers of either strip or discoidal shape, and addressed the contribution of E-cadherin on coordinated rotational motion. The applicant tracked movements of wild-type or E-cadherin knockout (KO) cells under two distinct boundary conditions, the strip and the disc, and analyzed their migrations. As far as the strip pattern was concerned, the KO cells migrated towards free space as fast as the wild-type cells. In contrast, on the disc, the wild-type cells exhibited persistent and large-scale rotations, while the E-cadherin KO cells migrated in a less coordinated manner and showed only transient local rotations. Direct quantitative comparison between these two cell types using particle image velocimetry (PIV) unveiled that the E-cadherin KO cells exhibited the following changes in migratory behaviors: (1) decreased global migration speed, (2) enhanced irregularity in quantified coordination and (3) increased average number of the topological defects. Collectively, under 2D discoidal confinements, these findings show that the emergence of large-scale coordinated migration of epithelia depends on E-cadherin.

It has not been addressed how the collective motion is affected when E-cadherin-deficient cells appear in epithelial monolayers from biophysical and pathological points of view. On the basis of the above results, the applicant attempted to conduct cell migration analysis by mixing wild-type and E-cadherin KO cells on the disc. Because the applicant intended to monitor intercellular dynamics at both homo- and hetero-interfaces in the cell mixture, visualization of the plasma membrane of individual cells became particularly important. To this end, the applicant established EGFP-CAAX or TagRFP-T-CAAX-expressing wild-type MDCK II clones. Unexpectedly, in contrast to the original wild-type cells, those plasma membrane-labeled wild-type cells did not initiate large-scale coordinated movements when cultured on the disc. The applicant discussed possible effects of the expression of the CAAX-fused fluorescent proteins on collective behaviors of cells.

(Form 2)

(Thesis Evaluation Summary)

The applicant reported that migratory manners between the two cell types (wild-type and E-cadherin KO MDCK II cells) became profoundly different on the discoidal pattern, which showed that E-cadherin is required for the rapid collective rotation, and discussed the experimental results in satisfactory manners. In the E-cadherin KO cells, it is likely that the decrease in the total cadherin activity in each cell does not allow the long-range intercellular force transmission. This possibility may be strengthened by visualizing distributions of the actin cytoskeleton and vinculin. Alternatively, E-cadherin may have a unique role in collective epithelial migration, which cannot be compensated by other cadherin subclasses including cadherin-6 expressed in MDCK II cells. Further comparative studies using wild-type cells, E-cadherin KO cells, and cadherin-6 KO cells would resolve these possibilities.

One feature of the adhesive disc in the applicant's study was its large size, compared to the discs employed in other studies and the intrinsic correlation length of MDCK cells. It can be addressed in future how differently E-cadherin KO cells behave on smaller discs where the applicant could reproduce clear rotational migration of wild-type cells. Another point of discussion concerning the experimental design was the broader definition of "collective migration" of cells on discs under the confluent proliferation-inhibited condition, in contrast to a classic view of collective cell migration where leader cells exert traction on the follower cells towards free space. Finally, effects of the CAAX-fused fluorescent proteins on the collective rotation was discussed in the context of additional experiments to further verify the applicant's hypothesis.

This thesis substantiates the candidate's extensive and wide knowledge of life sciences, demonstrates expert research capability in the field of cell biology, and presents new discoveries that contribute to the profound understanding and further development of the candidate's research field. Moreover, the thesis is written logically and coherently, which satisfies the degree requirement that the thesis shall serve as a valuable document for future reference. On April 13th, 2023, the PhD thesis oral examination was held. Pursuant to this oral examination, the thesis examination committee hereby concludes that the candidate has passed all of the requirements for the degree of Doctor of Philosophy in Life Sciences.