

Investigation of the Dynamics of Focal Adhesion Complexes on the Patterned Surfaces by Super-resolution Imaging

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It is known that cells can attach to the substrate surfaces through the binding to the extracellular matrix elements such as fibronectins and collagens forming focal adhesion complexes. Therefore, it is possible to manipulate the adhesion, migration and proliferation of cells on the substrate surfaces by patterning these extracellular matrix elements using micro-contact printing. It has been shown that the behavior of cells on the patterned surfaces did depend on the size of the patterned area on the micrometer scale. However, when the size of these patterns is reduced to the nanometer scale, it is not known that how cells will response to these patterned proteins because of the lack of proper tools. In recent years, the development of the stimulated emission depletion microscopy (STED), the photoactivated localization microscopy (PALM), the stochastic optical reconstruction microscopy (STORM), and the saturated structured illumination microscopy (SSIM), which improve the resolution of the optical microscopy by about ten folds compared to the conventional optical microscopy, makes it possible to investigate the behavior of living cells at nanometer scale.[1-3] Here, we report the investigation of the dynamic of focal adhesion complexes on the micro- and nano-patterned area by the photoactivated localization microscopy.

To utilize the photoactivated localization microscopy for the study of focal adhesion complexes, the CHO cells were transfected with a photo-activated protein, kaede, which was fused with a protein, paxillin, in the focal adhesion complexes. The CHO cells were cultured on the glass substrates patterned with array of fibronectins. The fluorescence images of living cells were measured on a total internal reflection fluorescence (TIRF) microscope using photo-activation sequences. The PALM images were reconstructed by the localization algorithm. Our preliminary results indicate that it is possible to observe the evolution of focal adhesions as well as individual paxillin molecules during the migration of CHO cells on the patterned surface using super-resolution microscopy.

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[3] Hari Shroff, Catherine G Galbraith, James A Galbraith, and Eric Betzig, "Live-cell photoactivated localization microscopy of nanoscale adhesion dynamics," *Nature Methods* 5, 417-423 (2008).