

## Wound Healing at Single Cellular Level Studied with Confocal Fluorescence Microscope

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Wound healing is an important biological process to protect organisms from deteriorations as after effects of bodily damages [1]. In this work we focused on the repair process of artificially inflicted injuries on the single cellular level by the means of nano-mechanical force [2]. For this purpose, AFM cantilever with a colloidal bead was coated with the enzyme, phospholipase A<sub>2</sub> (PLA<sub>2</sub>). Cells in culture were labeled with fluorescent Kusabira Orange (KO) using a vector having DNA sequences for KO and for a plasma membrane targeting peptide. In a similar manner, the cytoskeletal proteins ( $\beta$ -actins) were specifically labeled with green fluorescent protein (GFP). When the AFM probe was lifted after a short contact with the cell surface, a small non-fluorescent hole was detected under a confocal fluorescence microscope. This is the result of PLA<sub>2</sub> assisted degradation of membrane phospholipids. The non-fluorescent hole gradually recovered its fluorescence or never recovered it depending on the size of the hole. When holes were sealed (Figure 1(a)), an average resealing rate of smaller holes was determined as  $\sim 0.014 \mu\text{m}^2\text{s}^{-1}$  for the first time. A hole as large as  $10 \mu\text{m}$  in diameter seemed difficult to be repaired automatically (Figure 1(b)). The slow rate of hole resealing indicates that it is not a simple diffusion process of membrane lipids but most likely involves participation of vesicle fusion and cortical rearrangement. Mobilizations of focal-adhesion related proteins were also studied.

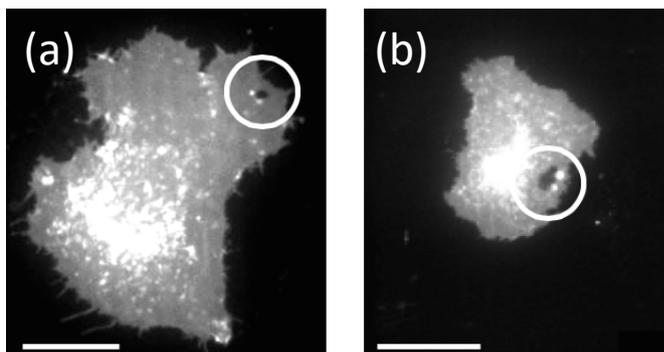


Figure 1. Holes in circles, re-sealed (a) and not re-sealed (b). KO labeled cells observed under confocal fluorescence microscope. Bars=40  $\mu\text{m}$ .

Many cells started to form “blebs”, i.e., bubble-like protrusions of the cell surface structures after hole creation. Blebs were formed near the newly created hole or all over the cell surface almost simultaneously. The structural information of hole creation must be transferred to even distant parts of the same cell.

### References:

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