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Cell-cell Interactions in Diseased Conditions Revealed by Three Dimensional and Intravital Two Photon Microscope: From Visualization to Quantification

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Extended Abstract

In the physiological and pathological conditions, complex cellular interplay takes place in living animals. However, conventional microscope using two dimensional analysis was not sufficient for analyzing in cell dynamics and functions in vivo. Thus, we developed in vivo imaging technique based on multi-photon microscopy to revealed the multicellular processes during thrombus development and artery contraction processes.

First, we visualized the cell dynamics including single platelet behavior, and assessed dynamic cellular interplay in two thrombosis models to elucidate the underlying cellular mechanisms of cadiovascular diseases. As a first model, thrombus formation was triggered by ROS photochemically induced by moderate power laser irradiation. In this model, thrombus consisted by discoid platelet aggregations without leukocyte recruitment. The second model is, thrombus with EC disruption. High power laser induced EC erosion and extravasations of circulating leukocytes with thrombus development. We developed new software which track and quantify the developing volume of thombus to validate our results.

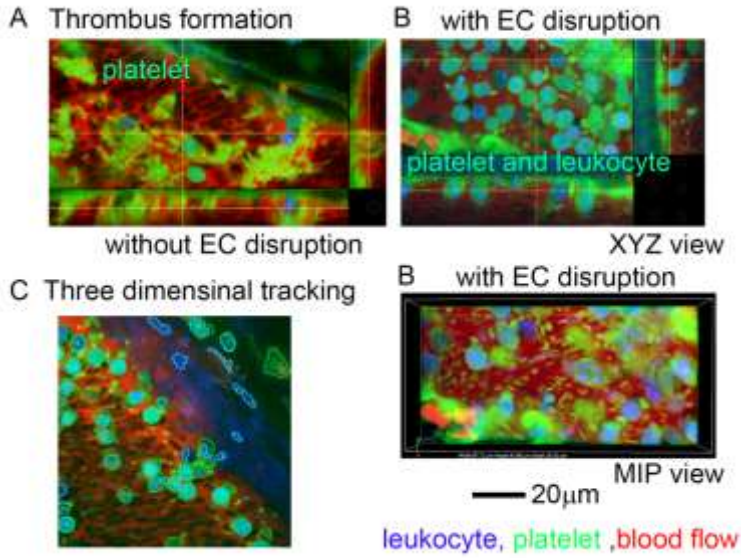

Fig.1. Intravital three dimensional visualization of thormbus formation.

In addition, we recently succeeded in visualizing femoral artery by three dimensional multi-photon microscope technique. We elucidated the artery contraction reactions against ROS stimulation. Using this novel animal model, we elucidated the functional contribution of smooth muscle cell of artery to

hypertensive diseases. We also directly measured the ROS and NO production in smooth muscle cells by fluorescent indicators, which was analyzed and quantified by novel tracking software.

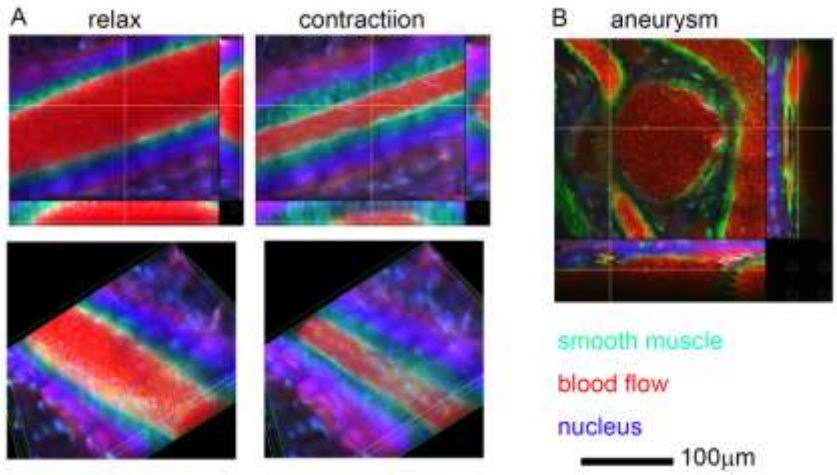

Fig.2. Visualization of artery contractions (A) and remodelling (B) in living mice.

In conclusions, three dimensional analysis of living mice using two photon microscope revealed the diseased conditions in single cell, and molecular levels.

References

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