RHOGOCYTE CELL ISOLATION AND CHARACTERIZATION FROM MOLLUSC’S TISSUE USING FACS IF-FISH

Faried Sairi 1,2, *, Peter Valtchev 1, Vincent Gomes 1, Fariba Dehghani 1,
1 School of Biochemical and Biomolecular Engineering, Faculty of Engineering and Information Technology, University of Sydney 2006 NSW, Australia
2 School of Bioscience and Biotechnology, Faculty of Science and Technology, Universiti Kebangsaan Malaysia, 43600 Bangi, Selangor, Malaysia
Fariba.dehghani@sydney.edu.au

Key words: Rhogocyte cell; Hemocyanin; Fluorescence-activated cell sorting (FACS); Immunofluorescence-fluorescence in situ hybridisation (IF-FISH), confocal microscope

Rhogocyte cells are known as the hemocyanin biosynthesis site, scattered within mollusc’s connective tissue. The protein immunogenic properties are extensively studied and used in biomedical application. In contrast to its application, knowledge for its biosynthesis is still obscure due to challenges in isolating and culturing the rhogocyte cells. In this study, we aim to solve the challenges of rhogocyte cells isolation and its characterization as a single cell. To achieve this, cells were isolated using Fluorescence-activated cell sorting (FACS) based on simultaneous staining of hemocyanin antibody and its mRNA probes (IF-FISH). Further observation with confocal microscopy was performed to characterize the cells before and after sorting. The results of this study demonstrated the isolation of two distinctive cells populations with overlapping signals. Both populations had varied cell morphology, sizes and IF-FISH signal distribution. The population with high antibody signal had irregular and elongated cell morphology with punctate mRNA probes signal. The second population with lower antibody had ovoid morphology and wide distribution of mRNA probes signals. Hemocyanin localization in the membrane pore structure was detected for both populations when observed by confocal microscopy, followed by mRNA probes signal in the cytoplasm. Thus, we confirmed the isolation of rhogocyte cells from mollusk using a combination of FACS and IF-FISH. The result of this study can be a turning point to further understand the mechanism of hemocyanin biosynthesis in vitro.