Development of a rapid quantitative ultrastructural evaluation of the blood platelet profile: with a provision of human iPS cell-derived platelets production for clinical transfusion

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Platelets, a component of blood, are responsible for blood clot formation to stop bleeding. They are fragments of cytoplasm, without cell nucleus, released from megakaryocytes in the bone marrow. Although 200-300 billion platelets are necessary in every clinical platelet transfusion, fresh single-donor platelets have a short shelf-life and readily lose clotting activity within several days. Most recently, clinically applicable generation of platelets are reported by using expandable megakaryocyte cell lines developed from human induced pluripotent stem (iPS) cells (Nakamura et al., Cell Stem Cell 14: 535 - 548, 2014). Because of the average of platelets diameter (reaching only 2 m), electron microscopy is necessary to evaluate the platelet profile consisting of granules, mitochondria, open canalicular system (OCS), glycogen granules, etc.

In this context, we developed a simple backscattered electron (BSE) imaging, instead of time-consuming transmission electron microscopy (TEM), using a scanning electron microscope (S-4800, Hitachi High-Technologies, Tokyo, Japan) followed by digital quantitative evaluations by means of a customized software, with a provision of human iPS cell-derived platelets production for clinical transfusion. The BSE imaging over epoxy resin embedded semi-thin (1-2 m in thickness) section provided high-resolution electron micrograph enough to distinguish the ultrastructure as small as 20 nm diameter glycogen granules within the platelets. Although the TEM requires skillful and time-consuming sample preparation, the total sample preparation for the present BSE imaging required less than five hours from primary fixation with glutaraldehyde to heavy metal (uranyl acetate and lead citrate) staining via improved rapid (= 2 hours) epoxy resin embedment.

Following the observation, the digital quantitative evaluations were "semi-automatically" performed using the customized "Image-Pro Premier" software produced by Media Cybernetics, Inc. (Rockville, Maryland, USA). Thus far, a conventional software takes 30 minutes to evaluate 20-30 platelets, but our preliminary examinations yields more than 500 platelets profiling within

2 minutes concerning their size (both major and minor axes), contour, area, etc. We introduced the development of rapid quantitative ultrastructural evaluation of the blood platelet profile that would also be useful for ultrastructural evaluation of the other regenerated organs developed from human iPS cells.

This work was (partially) supported by the Project Focused on Developing Key Evaluation Technology: Evaluation for Industrialization in the Field of Regenerative Medicine from Japan Agency for Medical Research and Development (AMED).