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Quantitative evaluation of morphological changes in activated platelets in vitro using digital holographic microscopy

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ABSTRACT

Platelet activation and aggregation have been conventionally evaluated using an aggregometer. However, this method is suitable for short-term but not long-term quantitative evaluation of platelet aggregation, morphological changes, and/or adhesion to specific materials. The recently developed digital holographic microscopy (DHM) has enabled the quantitative evaluation of cell size and morphology without labeling or destruction. Thus, we aim to validate its applicability in quantitatively evaluating changes in cell morphology, especially in the aggregation and spreading of activated platelets, thus modifying typical image analysis procedures to suit aggregated platelets. Freshly prepared platelet-rich plasma was washed with phosphate-buffered saline and treated with 0.1% $CaCl_2$. Platelets were then fixed and subjected to DHM, scanning electron microscopy (SEM), atomic force microscopy, optical microscopy, and flow cytometry (FCM). Tightly aggregated platelets were identified as single cells. Data obtained from time-course experiments were plotted two-dimensionally according to the average optical thickness versus attachment area and divided into four regions. The majority of the control platelets, which supposedly contained small and round platelets, were distributed in the lower left region. As activation time increased, however, this population dispersed toward the upper right region. The distribution shift demonstrated by DHM was essentially consistent with data obtained from SEM and FCM. Therefore, DHM was validated as a promising device for testing platelet function given that it allows for the quantitative evaluation of activation-dependent morphological changes in platelets. DHM technology will be applicable to the quality assurance of platelet concentrates, as well as diagnosis and drug discovery related to platelet functions.

1. Introduction

Recent developments and subsequent advancements in digital holographic microscopy (DHM) have enabled the continuous or intermittent observation of morphological changes in the same cells with regard to cell height, volume, surface roughness, surface irregularity, and many other indexes without destruction, fixation, or labeling (Molder et al., 2008; Kemper et al., 2010; Kim, 2010; Mir and Shinohara, 2012; Wang et al., 2013). In general, morphological changes in cells in vitro are microscopically examined using selected views. Consequently, given the limited number of cells subjected to qualitative evaluation, data obtained therefrom may not necessarily represent the overall trend in cell populations. DHM, however, allows the scanning of more than 1000 cells within minutes, as well as the quantitative evaluation of their morphology. Therefore, it has the advantage of being able to detect small but statistically significant differences among similar cell populations.

Nevertheless, notable limitations have also accompanied DHM. First, the resolution limit and other aspects, which are dependent on individual biological DHM types, have been optimized for most nucleated adherent cells, which have a diameter of 10 μm or larger. Moreover, the officially disclosed procedure for DHM (HoloMonitor M4; Phase Holographic Imaging, Lund, Sweden) suggests this device's lateral resolution of 1 μm, thereby making it unsuitable for examining small objects, such as platelets. Second, a common limitation in image analysis technology is the difficulty in using the automatic mode for the precise and quick analysis of cells that are tightly packed or horizontally overlapping. In such a case, operators need to carefully perform

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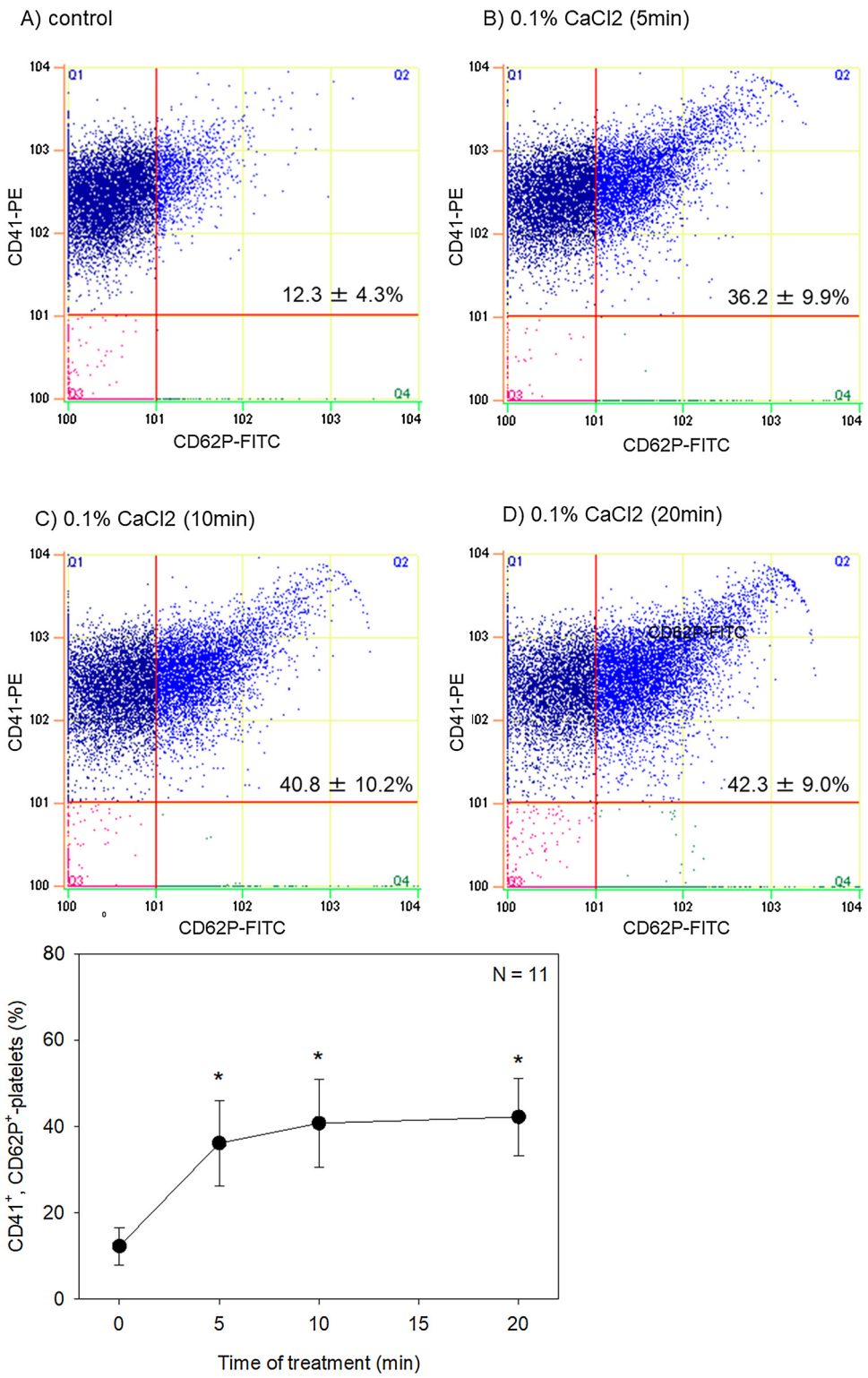

Fig. 1. Time-course changes in CD62P expression of single platelets. Washed platelets were treated with 0.1% $CaCl_2$ in sample tubes for (B) 5 min, (C) 10 min, or (D) 20 min, fixed and probed with FITC-conjugated anti-CD62P and PE-conjugated anti-CD41 antibodies. (A) Control (no addition). Percentages of $CD41^+/CD62P^+$ platelets were determined from the representative single donor sample. Similar data were obtained from three additional experiments using different donor samples. (E) Time-course changes in the percentage of $CD41^+/CD62P^+$ single platelets (N = 11). $^*P < 0.05$ versus control.

segmentation of the individual cells. Theoretically, however, vertically aggregated platelets cannot be segmented for image analysis. Therefore, if data from single platelets contained in aggregates are collected and analyzed, DHM may generally not be suitable for platelet examination.

In fact, the history of using DHM to study platelets is not long. To our knowledge, only a few pilot studies were published in 2015 (Boudejltia et al., 2015; Tamrin et al., 2015). These studies provided fundamental knowledge required for platelet examination, but did not indicate much about the differences between single resting platelets