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Supplementary Information

Fully automated light transmission aggregometry on a disc for platelet function tests

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Figure S1. Device assembly (the top, middle, and bottom layers of the computer-numerical control (CNC)-milled polycarbonate layers with the double-sided adhesive tape in between.



Figure S2. Robustness of the infrared sensor in three different light conditions measuring the optical density of a food dye at different dilution ratios.



Figure S3. Well-to-well reproducibility of measuring the absorbance of a food dye at different concentrations in three different wells (n=5). C.V.(%) at different concentrations are 0.63%, 4.35%, and 8.32%, respectively.



Figure S4. Disc-to-disc reproducibility of measuring the absorbance of a food dye at different concentrations with three different discs (n=3). C.V.(%) at different concentrations are 3.25%, 4.58%, and 8.73%, respectively.



Figure S5. The number of platelets distributed into each reaction well on the disc (2.89 \pm 0.36 \times 10⁵/ml) confirming that there was no significant difference in the platelet concentration distributed to each well of the disc (n=3). One-way ANOVA with Tukey's honestly significant difference (HSD) *post-hoc* test was used (p = 0.8954, ns).



Figure S6. The spinning profile of the agitation condition (0.61 m s–2 and 2 Hz). The spinning profile was repeated for 30 s.



Figure S7. Effect of spinning speed on data acquisition reliability.



Figure S8. Flow cytometry analysis for the activation level of PRP sample prepared by disc. The platelet activation is calculated by the percentage of CD62P positive platelets of the CD41 positive platelets.



Figure S9. The aggregation level using 32 μ M TRAP-6 at two different purity levels of platelets (n=3). A two-way ANOVA test was performed, and the p-value shown in the graph indicates the effects of the platelet purity. Statistical significance was indicated as follows; ****, p < 0.0001; ***, p < 0.001; **, p < 0.01; *, p < 0.05; ns, not significant.



Figure S10. The platelet aggregation without agonist was compared for the conventional method using a 96-well plate and on-disc operation (n=3). The mechanically triggered aggregation was $1.4 \pm 0.1\%$ per cycle for on-disc operations, with a linear progression up to $11 \pm 2\%$ of aggregation after the 10^{th} cycle. However, in the conventional method using 96-well plates, it showed a sigmoidal progression, with a large aggregation of $16 \pm 8\%$ for the initial cycle while reaching a plateau of $22 \pm 9\%$ aggregation after the 10^{th} cycle.



Figure S11. LTA assay with AA (1 mM) on 96-well plate and on-disc as a function of actual time after PRP and PPP preparation.



Figure S12. The aggregation level of PRP samples using 32 μ M TRAP-6 at two different concentrations of platelets (PLT-High: $1.1 \pm 0.1 \times 10^7$ /ml, PLT-Low: $3.7 \pm 0.4 \times 10^6$ /ml, n=3). The platelet concentrations were chosen to reflect the difference of the platelet concentration prepared by conventional methods (PLT-High) and on-disc (PLT-Low). A two-way ANOVA test was performed, and the p-value shown in the graph indicates the effects of the platelet concentration. Statistical significance was indicated as follows; ****, p < 0.0001; ***, p < 0.01; *, p < 0.05; ns, not significant.