VII. APPENDIX A

Name of reagent, commercial kit or chemical substances and equipment	Company
Reagents	
Adenosine 5' diphosphate (ADP)	Sigma
Collagen	Sigma
Epinephrine	The Government Pharmaceutical Organization (GPO)
Ristocetin	Sigma
Poly-L lysine	Sigma
Commercial kits	
Platelet factor 4 (PF4) ELISA kit	Diagnostica stago
β-Thromboglobulin (β-TG) ELISA kit	Diagnostica stago
Chemical substances	
Aspirin	Bayer®
Bovine serum albumin (BSA)	Sigma

Name of reagent, commercial kit or chemical substances and equipment	**Company**
Chemical substances	
Caffeine	Maxway
Calcium chloride ($CaCl_2$)	Merck
Ethylenediaminetetraacetic acid dipotassium dihydrate; EDTA ($C_{10}H_{14}K_2H_2O_8.2H_2O$)	Fluka
Glyoxal	Fluka
N-[2-hydroxyethy] piperazine-N [2-ethanesulfonic acid] (HEPES)	Sigma
N-ethyl maleimide (NEM)	Fluka
Paraformaldehyde	Sigma
37% Formaldehyde	Sigma
50% Glutaraldehyde for SEM	Sigma
Potassium chloride (KCl)	Merck
Potassium dihydrogen phosphate (KH_2PO_4)	Merck
Sodium chloride (NaCl)	Merck
Sodium azide (NaN_3)	Merck
Sodium dihydrogen phosphate dihydrate ($NaH_2PO_4.2H_2O$)	Merck
Tri sodium citrate dihydrate ($C_6H_5Na_3O_7.2H_2O$)	Merck
Theophylline	Merck

Name of reagent, commercial kit or chemical substances and equipment	**Company**
Recombinant protein	
Annexin V-FITC	Caltag
Monoclonal antibodies	
Anti CD42b-FITC	Caltag
Anti CD63-RPE	Caltag
Anti GPA-RPE	Caltag
Isotypc control IgG-FITC	Caltag
Isotype control IgG-RPE	Caltag
Equipment	
Automatic blood cell analyzer (Hemacel)	Hycell
Centrifuge (H-103RS)	KOKUSAN
Flow cytometer (FACSort)	Becton Dickinson
Scanning electron microscopy (JSM-840A)	JEOL

APPENDIX B

Protocol for preparation of reagents

1. 3.2% tri-sodium citrate (0.106 M)

Tri-sodium citrate dihydrate ($C_6H_5Na_3O_7.2H_2O$)	3.2	gm
Distilled water (DW; non ionic)	100	mL

The solution was filtered with filter paper (Whatman No.1) and kept at room temperature (RT).

2. Anti platelet mixture

Aspirin (3 tablets of 500 mg tablet)	1.5	gm
3.2% tri-sodium citrate solution	75	mL

The solutions stirred at RT overnight and then add;

Caffeine	0.625	gm
Theophylline	0.313	gm
NaN_3	0.1	gm

The solution was adjusted the final volume to 100 mL and stirred at RT for 2 hr. After that filtered with 0.45 μm membrane filter and kept in light protect bottle at RT.

3. Phosphate buffer saline (PBS) pH 7.3

NaCl	8.0	gm
KCl	0.2	gm
Na_2HPO_4	1.15	gm
KH_2PO_4	0.2	gm
DW (non ionic)	1000	mL

The pH was adjusted to 7.3, the solution for cell culture, must be filtered with 0.2 μm membrane filter or autoclaved at the condition of 121^{0}C with the pressure of 15 pound/inch^2 for 15 min. It was kept at RT.

4. Annexin V buffer (AVB)

HEPES	2.383	gm
NaCl	8.182	gm
$CaCl_2$	0.278	gm
NaN_3	0195	gm
Bovine serum albumin (BSA)	0.01	gm
DW (non ionic)	1000	mL

The solution was filtered with 0.45 μm membrane filter and kept at 4^{0}C.

5. 0.05% formaldehyde in AVB

37% formaldehyde	1	mL
AVB solution	739	mL

The solution was filtered with 0.45 μm membrane filter and kept at RT.

6. 0.2 M phosphate buffer pH 7.3

Stock solution 0.2 M Sodium phosphate monobasic (Solution A)

NaH_2PO_4. $2H_2O$	31.21	gm
DW (non ionic)	1000	mL

Stock solution 0.2 M Sodium phosphate dibasic (Solution B)

Na_2HPO_4. $2H_2O$	35.61	gm
DW (non ionic)	1000	mL

The solution was filtered with 0.45 μm membrane filter and kept at RT.

0.2 M phosphate buffer working pH 7.3

Solution A	230	mL
Solution B	770	mL

The solution was stirred at RT for 15 min and kept at RT

0.1 M phosphate buffer working pH 7.3

0.2 M phosphate buffer	100	mL
DW (non ionic)	100	mL

The solution was kept at RT.

7. 0.25% glutaraldehyde in 0.2 M phosphate buffer pH 7.3

50% glutaraldehyde	100	μL
0.2 M phosphate buffer	19.9	mL

8. 2% glutaraldehyde in 0.1 M phosphate buffer pH 7.3

50% glutaraldehyde	800	μL
0.1 M phosphate buffer	19.2	mL

9. 0.2% glyoxal with 0.4% paraformaldehyde fixative solution

0.4% glyoxal

40% glyoxal	1	mL
PBS pH7.3	99	mL

0.8% paraformaldehyde

Paraformaldehyde	0.8	gm
PBS pH 7.3	100	mL

Mixture solution of 0.2% glyoxal with 0.4% paraformaldehyde consisted of 0.8% paraformaldehyde 100 mL and 0.4% glyoxal 100 mL and stirred at RT for 15 min. It was kept in light protect bottle at 4^0C.

10. Incubation buffer

Tris /HCl	1.211	gm
DW (non ionic)	1000	mL

The solution was adjusted pH to 7.4 by 2 N HCl. It was filtered with 0.45 μm membrane filter and kept at RT.

11. 10 mM N-ethyl maleimide (NEM)

NEM	0.125	gm
Incubation buffer	100	mL

The solution was kept at RT.

12. 2.5 mM EDTA

EDTA	0.101	gm
Incubation buffer	100	mL

The solution was kept at RT.

13. CPDA-1-0.1% BSA-PBS pH 7.3

0.1%BSA-PBS pH 7.3

BSA	1	gm
PBS pH7.3	1000	mL

The solution was filtered with 0.45 μm membrane filter and kept at 4^0C

CPDA-1-0.1%BSA-PBS pH 7.3

CPDA-1 (solution)	10	mL
0.1% BSA-PBS pH 7.3	70	mL

The solution was kept at 4^0C

14. 1% formaldehyde in CPDA-1-0.1% BSA-PBS pH 7.3

37% formaldehyde	1	mL
CPDA-1-0.1% BSA-PBS pH 7.3	36	mL

The solution was kept at 4^0C

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