ONLINE SUPPLEMENTARY CONTENT

Table SI - The results of BCA protein assay and TRPS in exosome samples and the exosome levels in non-irradiated and irradiated apheresis platelet concentrates samples

				Exosom	e samples				
Total protein amount and number of exosomes			Non-irradiated		Irradiated				
	Day 0 Day 5		p-value	Day 0	Day 5	p-value			
	Median	5.46	4.77		5.19	4.58	0.959		
BCA Assay (μg/μL)	Q1	4.95	4.56	0.333	4.97	4.21			
	Q3	6.46	5.17		5.71	5.12	I		
BCA Assay (μg/μL)	(Pool)	5,07	4,83		5,13	4,74			
TRPS (Particle/μL)	μ L) (Pool) 8,9E+07 8,7E+07			11,1E+07	8,2E+07				
		Exosome samples							
Exosome levels		Non-irradiated			Irradiated				
	Day 0	Day 5	p-value	Day 0	Day 5	p-value			
	Median	98%	98%		98%	98%	0.623		
Total Exosome (CD9*)	Q1	97%	95%	0.208	94%	95%			
	Q3	99%	99%		99%	99%			
Platelet Derived Exosome (CD9 ⁺ 41 ⁺)	Median	87%	84%		84%	85%	0.924		
	Q1	82%	81%	0.049	79%	84%			
	Q3	91%	88%		89%	88%			
	Median	5%	8%		7%	7%			
Granulocyte Derived Exosome (CD9*15*)	Q1	2%	5%	0.049	2%	4%	0.484		
	Q3	7%	11%		12%	11%			

Sample numbers are 8 for each column (except pooled exosome columns).

BCA Protein Assay was used to determine the total amount of protein in the isolated and pooled exosome samples. TRPS was used to detect the number of exosomes in the pooled samples. The exosome levels were not affected by the storage time and gamma-irradiation. In the non-irradiated group, the decrease in PD-EX levels on the storage day 5 compared to day 0 and the increase in GD-EX levels are statistically significant. PD-EX: platelet derived exosome, GD-EX: granulocyte derived exosome, TRPS: tunable resistive pulse sensing. PD-EX: platelet derived exosome, GD-EX: granulocyte derived exosome. p-values <0.05 was considered statistically significant; significant changes are shown in bold. The levels of exosomes expressed from cells other than platelets and granulocytes (from red blood cells, lymphocytes, monocytes, NK cells, NK-T cells and myeloid-derived suppressor cells) remained in the range of 0-2%. Results in the range of 0-2% were ignored as they varied within a very narrow range.

Table SII – The effects of ADP and collagen on maximum platelet aggregation (A) and the impacts of exosomes on maximum platelet aggregation and hemostasis (B)

(A)		Saline	NI.0	PC	Saline	IR.0	PC	Saline	NI.5	PC	Saline	IR.5	PC
ADP (2 μM)	37,8%	43,0%	0,14	74,4%	74,4%	0,00	21,6%	22,8%	0,06	21,6%	22,6%	0,05
Collagen (19 μg/mL)	40,7%	33,7%	-0,17	69,8%	71,0%	0,02	61,2%	62,2%	0,02	61,2%	66,4%	0,08
ADP (4 μM)	40,8%	40,8%	0,00	71,6%	79,8%	0,11	80,8%	79,8%	-0,01	67,0%	59,4%	-0,11
Collagen (38 μg/mL)	40,8%	41,8%	0,02	78,6%	70,0%	-0,11	73,4%	71,6%	-0,02	64,6%	61,8%	-0,04
ADP (10 μl	4)	47,8%	48,4%	0,01	67,6%	77,4%	0,14	70,4%	75,8%	0,08	68,0%	74,6%	0,10
Collagen (•	51,8%	47,8%	-0,08	76,8%	74,4%	-0,03	82,0%	72,2%	-0,12	78,6%	72,8%	-0,07
		Saline	NI.0		Saline	IR.0		Saline	NI.5		Saline	IR.5	
(B)			pool	р		pool	р		pool	р	Saune	pool	р
MPA (%) (n=8)	Median	60.0%	60.8%	0.108	59.7%	59.9%	0.343	60.0%	59.4%	0.396	59.1%	59.1%	0.128
	Q1	57.0%	52.4%		57.0%	53.6%		51.8%	53.4%		44.2%	42.4%	
	Q3	72.2%	68.6%		67.4%	67.6%		72.2%	69.4%		67.4%	68.2%	
R (Minute) (n=7)	Median	5.8	3.8		6.4	4.8		6.0	4.7	Y	5.0	4.6	
	Q1	8.2	6.3	0.018	7.5	6.4	0.018	6.0	5.7	0.063	7.0	7.2	0.060
	Q3	7.3	6.9		8.1	7.5		7.2	6.2		5.8	5.2	
α-Angle (Degree) (n=7)	Median	70.7	69.7		67.1	69.6		67.3	71.7		71.4	70.6	
	Q1	61.8	62.5	0.091	55.6	61.2	0.018	52.6	64.8	0.018	59.8	57.8	0.352
	Q3	69.1	69.2		66.2	67.5		66.3	70.8		71.9	74.8	
MA (mm) (n=7)	Median	72.1	70.6	0.237	69.8	72.1	0.933	67.6	72.4	0.150	69.3	64.7	0.799
	Q1	62.2	63.2		60.9	61.4		56.5	62.2		57.7	62.9	
	Q3	74.5	73.7		73.7	73.0		73.5	74.7		74.0	74.2	
G (K) (d/sc) (n=7)	Median	13.0	12.0		11.5	13.0		10.4	13.1		11.3	9.2	
	Q1	8.2	8.6	0.611	7.8	8.0	0.933	6.5	8.2	0.128	6.8	8.5	0.866
	Q3	14.6	14.0	<u>C</u>	14.0	13.5		13.9	14.8		14.2	14.4	
LY30 (%) (n=7)	Median	1.6	0.7		0.5	1.4		1.6	1.1		1.9	4.4	
	Q1	1.1	1.2	0.449	2.2	2.1	0.933	0.2	2.5	0.686	2.7	0.6	0.599
	Q3	0.5	0.8	ソ	1.4	0.0		0.1	0.1		0.8	1.3	
CI (n=7)	Median	2.1	3.2		1.1	2.6		1.1	3.0		2.3	2.0	
	Q1	-1.6	-0.2	0.018 -1	-1.9	-0.6	0.018	-2.0	0.4	0.018	-1.6	-1.2	0.063
	Q3	1.2	1.4		0.4	0.8		0.9	2.1		2.5	3.2	

⁽A) Whereas (1×) and (5×) ADP made the MPA increase, (5×) Collagen caused it to decrease. (1×) Collagen and (2×) ADP/Collagen led to both effects on MPA. MPA: Maximum platelet aggregation; PC: Percentage change.

⁽B) No significant difference was found among MPA values. R values significantly decreased in the NI.0 and IR.0 groups (p=0.018); α-angle values significantly increased in the NI.5 and IR.0 groups (p=0.018); CI values significantly increased in the NI.0. NI.5 and IR.0 groups (p=0.018) compared to saline groups. p-values <0.05 are considered as statistically significant and significant changes were shown in bold.

The platelet counts in the PRPs were measured as 444 (359-453) K/ μ L. Pooled exosome volumes or quantities or numbers used in aggregometry were calculated as 17.8 (14.4-18.1) μ L or 90.1 (72.8-91.9) μ g or 1.57E+09 (1.34E+09-2.15E+09) particles, respectively.

The platelet counts in the whole blood samples were measured as 188 (177-201) K/ μ L. Pooled exosome volumes or quantities or numbers used in thromboelastography were calculated as 15.4 (14.5-16.5) μ L or 78.2 (73.6-78.2) μ g or 1.57E+09 (1.31E+09-2.78E+09) particles, respectively.