

APPENDIX

Estimation of the volume and weight of GCF samples

Volume estimation

After preparing the filter paper strip in 2 mm X 10 mm size, human serum was used instead of GCF to measure the volume. An adjustable micropipette drew the different volumes of serum, i.e. 0.1, 0.2, 0.3, 0.4, 0.5 μl and then dropped each known volume on the 2 mm X 10 mm paper strip. The serum would migrate along the paper strip until it stopped. The distance of serum migration was measured by a caliper. In each volume, repeated trials were done for 30-40 times, and then the mean distance for each known volume was calculated. For approximately 2.0 mm long on 2 X 10 mm filter paper strip, the volume of serum was 0.4 μl .

Weight estimation

Fifty filter paper strips in 2 mm X 2 mm size were prepared, and then measured for their total weight. One paper strip was then calculated for its weight by dividing by 50 (supposedly by its value was A). The 2 mm X 10 mm filter paper strips were placed on human serum and the last 2.0 mm of filter paper strip containing the serum was cut. Fifty pieces of serum-containing filter paper strips were measured for the total weight. Then the weight of one piece of serum-containing paper strip was calculated by dividing by 50 (supposedly its value was B). The weight of serum in one 2.0 X 2.0 mm filter paper strip was obtained by subtracting A from B. From this method, the weight of serum that contained in one 2.0 X 2.0 mm filter paper strip was 53.9 μg .

The values of the Protein levels, WF6 epitope levels, and WF6 epitope levels per unit protein in GCF collected from all teeth were shown in Tables A.1-A.4.

Table A.1 Protein levels, WF6 epitope levels, WF6 epitope levels per unit protein in GCF samples from tooth C11 and C13 (N/A = not available), xxx means the time when an orthodontic force was applied to the canine.

Tooth	C11			C13		
	Time point	Protein	WF6 epitope /Protein	Protein	WF6 epitope	WF6 epitope /Protein
T0	N/A	N/A	N/A	51.43	755.16	14.68367
L0	1.00	61.562	61.562	8.21	1510.7	183.9113
L1	N/A	N/A	N/A	N/A	N/A	N/A
L2	N/A	N/A	N/A	N/A	N/A	N/A
L3	N/A	N/A	N/A	N/A	N/A	N/A
L4	1.00	389.03	389.03	14.64	5867.3	400.69366
L5	N/A	N/A	N/A	N/A	N/A	N/A
L6	N/A	N/A	N/A	N/A	N/A	N/A
L7	N/A	N/A	N/A	N/A	N/A	N/A
L8	1.00	169.38	169.38	1.00	1252.7	1252.7
L9	N/A	N/A	N/A	N/A	N/A	N/A
L10	N/A	N/A	N/A	N/A	N/A	N/A
L11	N/A	N/A	N/A	N/A	N/A	N/A
M0xxx	1.00	81.787	81.787	10.71	1630.1	152.14267
M1	1.00	130.49	130.49	1.00	406.63	406.63
M2	1.00	179	179	21.07	9478.3	449.81763
M3	1.00	232.4	232.4	1.00	150.15	150.15
M4xxx	1.00	452.68	452.68	1.00	356.69	356.69
M5	1.00	125.94	125.94	1.00	26.713	26.713
M6	N/A	N/A	N/A	N/A	N/A	N/A
M7	N/A	N/A	N/A	N/A	N/A	N/A
M8xxx	1.00	120.46	120.46	1.00	1755.5	1755.5
M9	1.00	161.29	161.29	1.00	111.71	111.71
M10	1.00	53.967	53.967	1.00	336.66	336.66
M11	1.00	60.811	60.811	1.00	163.69	163.69
M12xxx	3.83	99.154	25.866261	1.00	119.64	119.64
M13	1.00	443.38	443.38	1.00	220.74	220.74
M14	N/A	N/A	N/A	N/A	N/A	N/A
M15	N/A	N/A	N/A	N/A	N/A	N/A

Table A.1 (Continued)

Tooth	C11			C13		
	Time point	Protein	WF6 epitope WF6 epitope /Protein	Protein	WF6 epitope	WF6 epitope /Protein
M16xxx	1.00	52.633	52.633	1.00	1710.6	1710.6
M17	5.50	143.08	26.014545	1.00	87.177	87.177
M18	1.00	16.17	16.17	1.00	41.308	41.308
M19	1.00	17.16	17.16	1.00	357.42	357.42
S3	1.00	323.53	323.53	1.00	256.94	256.94
S4	10.08	11.137	1.1044959	1.00	209.96	209.96
S5	1.00	401.11	401.11	1.00	N/A	N/A
S6	1.75	352.87	201.64	5.36	150.52	28.097067
S7	1.00	95.028	95.028	1.00	32.234	32.234
S8	1.00	131.66	131.66	1.00	166.59	166.59
S9	1.00	13.129	13.129	1.00	136.66	136.66

Table A.2 Protein levels, WF6 epitope levels, WF6 epitope levels per unit protein in GCF samples from tooth D11, D13 and D23 (N/A = not available), xxx means the time when an orthodontic force was applied to the canine.

Tooth	D11			D13			D23		
Time point	Protein	WF6 epitope	WF6 epitope /Protein	Protein	WF6 epitope	WF6 epitope /Protein	Protein	WF6 epitope	WF6 epitope /Protein
T0	N/A	N/A	N/A	6.00	346.60	57.77	2.69	193.10	71.77
L0	N/A	N/A	N/A	61.38	129.10	2.10	8.76	4235.5	483.40
L1	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
L2	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
L3	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
L4	26.74	557.91	20.86	7.15	320.15	44.75	29.48	5498.6	186.54
L5	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
L6	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
L7	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
M0xxx	8.28	171.19	20.67	56.00	964.38	17.22	25.19	2154.6	85.53
M1	25.97	331.67	12.76	61.00	8416.90	137.98	11.26	3043.9	270.28
M2	11.74	423.4	36.05	54.46	10600	194.63	32.33	6674.1	206.42
M3	7.51	142.03	18.90	36.77	10300	280.13	10.19	269.87	26.48
M4xxx	10.97	1462	133.21	28.69	12800	446.11	44.83	9268.5	206.73
M5	17.90	369.17	20.62	20.62	3288.40	159.51	43.76	9609.6	219.59
M6	9.05	357.55	39.50	61.77	9976.90	161.52	17.33	1091.2	62.95
M7	3.28	145.14	44.22	25.62	9288.70	362.62	11.26	633.55	56.26
M8xxx	2.13	98.86	46.45	9.08	2207.00	243.14	8.76	686.89	78.40
M9	1.00	53.78	53.78	1.38	133.80	96.63	4.12	1113.0	270.21
M10	5.59	403.45	72.17	52.15	1129.60	21.66	10.55	4417.6	418.82
M11	10.59	468.93	44.28	36.77	8346.90	227.01	9.12	476.82	52.29
M12	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
M13xxx	41.74	6026.60	144.37	133.31	5646.00	42.35	81.26	11600	142.75
M14	11.36	228.18	20.09	6.00	184.27	30.71	21.62	747.31	34.57
M15	34.44	460.25	13.37	21.38	22.06	1.03	55.90	256.94	4.60
M16	6.74	428.75	63.58	87.92	13500	153.54	19.83	4441.2	223.93
M17xxx	2.13	33.11	15.56	15.23	8933.00	586.51	20.90	4446.3	212.69
M18	2.90	81.08	27.98	4.46	1077.50	241.51	21.98	6302.4	286.78
M19	0.97	101.06	103.72	2.54	776.68	305.96	13.05	6109	468.21
M20	2.13	151.09	70.99	29.85	824.25	27.62	18.76	5146.6	274.31
M21xxx	5.59	396.71	70.97	18.31	5092.10	278.14	35.19	9998.8	284.13

Table A.2 (Continued)

Tooth	D11			D13			D23		
	Time point	WF6 Protein	WF6 epitope	WF6 epitope /Protein	WF6 Protein	WF6 epitope	WF6 epitope /Protein	WF6 Protein	WF6 epitope
M22	1.00	75.30	75.30	26.77	8612.2	321.72	11.98	1283.3	107.15
M23	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
M24	1.00	51.73	51.73	8.69	3896.8	448.30	9.48	901.41	95.12
M25xxx	2.90	315.53	108.90	3.69	2470.7	669.15	33.40	586.09	17.55
M26	1.00	96.08	96.08	37.54	2965.7	79.00	29.12	2200.9	75.58
M27	1.95	134.96	69.26	12.15	1666.7	137.13	64.83	7112.7	109.71
M28	1.00	95.28	95.28	1.77	574.31	324.61	29.48	6662.9	226.04
M29	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
M30xxx	1.56	67.35	43.06	1.00	682.39	682.39	16.98	5992.4	352.99
M31	3.10	16.04	5.17	11.77	6055.4	514.51	37.33	5019.5	134.45
M32	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
M33	2.72	30.58	11.25	1.00	271.44	271.44	0.55	302.87	553.07
M34xxx	3.87	N/A	N/A	19.46	2812	144.49	63.76	8803.2	138.06
M35	8.87	94.71	10.68	1.00	3043.5	3043.50	85.19	8683.5	101.93
M36	12.33	174.63	14.16	36.77	3436.4	93.46	13.40	1834.7	136.87
M37	11.18	203.23	18.18	72.18	6281.5	87.03	8.86	295.58	33.36
M38xxx	5.41	N/A	N/A	8.33	49.08	5.89	17.61	5812.5	330.05
M39	18.49	486.35	26.31	58.33	12000	205.71	53.03	11500	216.87
S0	1.56	48.37	30.93	20.64	7086.7	343.33	15.94	N/A	N/A
S1	4.26	17.43	4.10	54.49	875.99	16.08	36.36	13900	382.28
S2	1.00	64.28	64.28	11.41	4599.4	403.09	33.86	3519.7	103.95
S3	6.56	81.19	12.37	52.95	10200	192.64	31.36	11200	357.13
S4	20.79	251.09	12.07	24.87	7258.3	291.83	13.03	434.39	33.34
S5	29.26	33.31	1.14	5.26	6225.4	1184.34	8.03	441.25	54.97
S6	15.79	162.78	10.31	12.56	7717.9	614.28	1.00	40.75	40.75
S7	5.79	48.55	8.38	24.49	3756.8	153.42	11.36	9488.7	835.19
S8	9.64	396.22	41.10	20.26	12200	602.28	1.00	N/A	N/A
S9	8.87	152.88	17.23	26.41	8261.9	312.83	26.36	9165.9	347.71

Table A.3 Protein levels, WF6 epitope levels, WF6 epitope levels per unit protein in GCF samples from tooth E11, E13 and E23 (N/A = not available), xxx means the time when an orthodontic force was applied to the canine

Tooth	E11			E13			E23		
	Time point	Protein	WF6 epitope	WF6 epitope /Protein	Protein	WF6 epitope	WF6 epitope /Protein	Protein	WF6 epitope
T0	N/A	N/A	N/A	1.00	2192.20	2192.20	1.00	913.09	913.09
L0	19.69	1326.00	67.34	31.45	5365.40	170.58	1.00	2145.00	2145.00
L1	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
L2	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
L3	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
L4	3.54	173.65	49.08	N/A	N/A	N/A	N/A	N/A	N/A
L5	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
L6	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
L7	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
L8	1.23	23.38	19.00	N/A	N/A	N/A	N/A	N/A	N/A
L9	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
L10	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
L11	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
M0xxx	7.38	2414.40	326.95	8.27	1239.00	149.77	1.00	495.58	495.58
M1	3.54	412.91	116.69	12.36	6702.10	542.08	113.38	20200.00	178.15
M2	6.23	726.53	116.60	4.18	1268.50	303.34	1.08	40.36	37.47
M3	2.38	30.30	12.71	1.00	199.84	199.84	1.00	101.75	101.75
M4xxx	47.00	1399.90	29.79	1.00	774.79	774.79	1.00	4251.70	4251.70
M5	5.46	346.16	63.38	15.09	2505.70	166.04	27.23	12200.00	448.02
M6	1.00	227.93	227.93	9.64	8010.80	831.31	14.92	15000.00	1005.15
M7xxx	1.00	27.50	27.50	12.36	14400.00	1164.71	7.62	20700.00	2718.18
M8	17.77	4485.00	252.40	1.00	888.05	888.05	26.85	12200.00	454.44
M9	1.00	120.82	120.82	24.64	12900.00	523.62	8.38	550.87	65.70
M10	6.62	402.15	60.75	8.73	4773.10	546.92	8.38	5499.90	655.95
M11xxx	1.00	26.59	26.59	7.82	2349.20	300.48	17.62	5063.40	287.44
M12	N/A	N/A	N/A	33.73	155.98	4.62	30.69	5.55	0.18
M13	46.23	47.83	1.03	34.64	2706.40	78.14	45.31	17600.00	388.46
M14	1.00	45.62	45.62	9.18	2654.00	289.05	30.69	12600.00	410.53
M15xxx	1.00	141.86	141.86	1.00	481.88	481.88	11.46	1593.60	139.04
M16	1.00	29.25	29.25	7.36	829.49	112.65	4.92	599.86	121.85
M17	7.38	776.17	105.11	8.73	3465.60	397.10	18.38	223.81	12.17
M18	1.00	34.34	34.34	6.91	930.55	134.68	12.23	127.35	10.41

Table A.3 (Continued)

Tooth	E11			E13			E23		
	Protein	WF6 epitope	WF6 epitope /Protein	Protein	WF6 epitope	WF6 epitope /Protein	Protein	WF6 epitope	WF6 epitope /Protein
M19xxx	1.00	125.04	125.04	1.00	N/A	N/A	21.08	8413.30	399.17
M21	5.46	61.42	11.25	10.55	3050.00	289.22	6.08	1016.10	167.21
M22	2.77	41.87	15.12	6.00	56.48	9.41	1.00	296.31	296.31
M23xxx	1.00	N/A	N/A	3.73	199.02	53.40	6.08	4126.80	679.09
M24	1.00	N/A	N/A	69.64	220.76	3.17	101.46	15.80	0.16
M25	8.92	625.43	70.09	46.00	2304.50	50.10	N/A	N/A	N/A
M26	9.69	88.97	9.18	4.18	20.25	4.84	N/A	N/A	N/A
M27xxx	7.38	55.53	7.52	62.82	159.87	2.54	N/A	N/A	N/A
S0	1.00	N/A	N/A	1.00	46.58	46.58	13.38	9826.00	734.13
S1	1.00	10.71	10.71	6.45	3526.40	546.34	9.54	946.78	99.26
S2	N/A	N/A	N/A	4.64	1247.60	269.09	31.08	454.47	14.62
S3	N/A	N/A	N/A	17.82	33.41	1.87	2.23	48.46	21.73
S4	1.00	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
S5	N/A	N/A	N/A	8.73	531.68	60.92	18.38	6173.60	335.80
S6	N/A	N/A	N/A	53.73	14900.00	277.33	23.77	3347.10	140.82
S7	N/A	N/A	N/A	1.00	717.25	717.25	31.08	1329.10	42.77
S8	1.00	23.68	23.68	1.00	19100.00	19100.00	3.77	N/A	N/A
S9	N/A	N/A	N/A	N/A	N/A	N/A	21.85	1401.00	64.13

Table A.4 Protein levels, WF6 epitope levels, WF6 epitope levels per unit protein in GCF samples from tooth H11, H13 and H23 (N/A = not available), xxx means the time when an orthodontic force was applied to the canine

Tooth	H11			H13			H23		
	Time point	Protein	WF6 epitope	WF6 epitope /Protein	Protein	WF6 epitope	WF6 epitope /Protein	Protein	WF6 epitope
T0	N/A	N/A	N/A	1.00	257.00	257.00	2.00	1668.70	834.35
L0	41.04	7453.90	181.64	35.51	6417.37	180.72	80.85	7081.50	87.59
L1	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
L2	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
L3	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
L4	39.93	60.30	1.51	13.70	4382.36	319.88	33.92	N/A	N/A
L5	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
L6	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
L7	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
M0xxx	1.00	23.07	23.07	15.30	11498.26	751.52	14.31	1492.10	104.29
M1	9.37	0.00	0.00	11.73	1297.22	110.59	7.38	216.47	29.31
M2	7.15	22900.00	3203.63	6.40	2688.00	420.00	63.54	391.98	6.17
M3	18.81	25700.00	1365.94	4.80	2465.09	513.56	45.08	3070.50	68.12
M4xxx	1.00	0.00	0.00	92.30	25.84	0.28	15.46	1574.60	101.84
M5	1.00	246.15	246.15	11.20	1470.56	131.30	8.92	375.48	42.08
M6	1.04	2254.00	2173.50	1.00	414.80	414.80	1.00	35.13	35.13
M7	1.00	128.67	128.67	1.00	70.17	70.17	1.00	138.22	138.22
M8xxx	1.00	0.00	0.00	12.80	274.05	21.41	N/A	N/A	N/A
M9	1.00	0.00	0.00	1.00	230.78	230.78	1.00	110.05	110.05
M10	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
M11	5.48	10300.00	1879.05	1.00	683.94	683.94	1.00	37.37	37.37
M12xxx	1.00	48.33	48.33	8.01	3168.84	395.61	5.08	202.99	39.98
M13	1.00	790.90	790.90	1.00	396.52	396.52	1.00	46.85	46.85
M14	9.37	5836.70	622.89	1.00	211.93	211.93	1.00	74.09	74.09
M15	1.00	0.00	0.00	1.00	186.99	186.99	1.00	207.71	207.71
S0	1.00	2236.60	2236.60	1.00	74.36	74.36	1.00	36.71	79.54
S1	1.00	259.19	259.19	1.00	95.56	95.56	1.00	5461.40	186.35
S2	16.04	15800.00	985.22	1.00	51.07	51.07	0.46	60.91	1.18
S3	49.37	0.00	0.00	1.00	567.67	567.67	29.31	4867.10	180.26
S4	1.00	12700.00	12700.00	2.64	1334.15	505.36	51.62	4221.50	163.33
S5	1.00	205.74	205.74	1.00	856.05	856.05	27.00	156.49	156.49

Table A.4 (Continued)

Tooth	H11	H13	H23	Tooth	H11	H13	H23	Tooth	H11
Time point	Protein	WF6 epitope	WF6 epitope /Protein	Time point	Protein	WF6 epitope	WF6 epitope /Protein	Time point	Protein
S6	1.00	68.46	68.46	1.00	275.73	275.73	25.85	130.37	27.78
S7	1.00	N/A	0.00	1.00	64.21	64.21	1.00	17.74	8.87
S8	1.00	196.40	196.40	1.00	47.49	47.49	4.69	115.12	115.12

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CURRICULUM VITAE

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