

APPENDIX

Estimation the volume and weight of GCF samples

Volume estimation

2x10 mm filter paper strips (Whatman®) were prepared, and human serum was used instead of GCF to measure the volume. An adjustable micropipette was used to aspirate the different volumes of serum, i.e. 0.1, 0.2, 0.3, 0.4, 0.5 μl , and then drop each known volume of human serum onto the 2x10 mm filter paper strip. The serum migrated along the filter paper strip until it stopped. The distance of migration was measured by a caliper. In each volume, repeated trials were done for about 30-40 times, and then the mean distance was calculated for each known volume. For a 2x2 mm filter paper strip, the volume of serum was approximately 0.4 μl .

Weight estimation

2x2 mm filter paper strips (Whatman®) were prepared for 50 pieces, and then measured for the total weight of these 50 pieces. The weight of one free paper strip was then calculated (supposedly its value was A). The 2x10 mm filter paper strips absorbed human serum and were cut into 2x2 mm of filter paper strips which contained the serum. Fifty pieces of serum-containing filter paper strips were then measured for their total weight, and the weight of one piece of serum-containing paper strip was then calculated (supposedly its value was B). The weight of serum in a 2x2 mm filter paper strip was obtained by subtracting calculate A from B. From this calculation, the weight of serum that contained in a 2x2 mm filter paper strip was approximately 53.9 μg .

The values of the Protein levels, HA levels, and HA level/total protein of GCF samples were shown in Tables A.1-A.4.

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

Tooth	C11			C13			
	Time point	Protein	HA	HA/Protein	Protein	HA	HA/Protein
T0		N/A	N/A	N/A	N/A	N/A	N/A
L0		1.00	38.07	38.07	8.21	404.36	49.23
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	109.78	109.78	14.64	340.39	23.25
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	46.48	46.48	1.00	210.71	210.71
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	54.68	54.68	10.71	115.31	10.76
M1		1.00	71.92	71.92	1.00	37.38	37.38
M2		1.00	54.68	54.68	21.07	392.49	18.63
M3		1.00	74.53	74.53	1.00	22.94	22.94
M4xxx		1.00	111.41	111.41	1.00	29.01	29.01
M5		1.00	62.66	62.66	1.00	26.86	26.86
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	68.91	68.91	1.00	112.72	112.72
M9		1.00	49.77	49.77	1.00	84.50	84.50
M10		1.00	55.88	55.88	1.00	54.31	54.31
M11		1.00	41.58	41.58	1.00	59.12	59.12
M12xxx		3.83	101.03	26.36	1.00	35.58	35.58
M13		1.00	89.76	89.76	1.00	45.90	45.90
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
M16xxx		1.00	25.67	25.67	1.00	176.84	176.84

Table A.1 (Continued)

Tooth	C11			C13		
	Protein	HA	HA/Protein	Protein	HA	HA/Protein
M17	5.50	62.81	11.42	1.00	32.81	32.81
M18	1.00	37.21	37.21	1.00	9.35	9.35
M19	1.00	28.31	28.31	1.00	59.25	59.25
M20xxx	1.00	21.45	21.45	21.07	77.24	3.67
S0	1.00	81.77	81.77	4.64	169.06	36.41
S1	1.00	60.75	60.75	1.00	50.32	50.32
S2	1.00	69.74	69.74	1.00	64.47	64.47
S3	1.00	75.24	75.24	1.00	22.13	22.13
S4	10.08	25.52	2.53	1.00	66.45	66.45
S5	1.00	75.96	75.96	1.00	14.72	14.72
S6	1.75	75.78	43.30	5.36	60.46	11.29
S7	1.00	36.73	36.73	1.00	20.81	20.81
S8	1.00	64.33	64.33	1.00	62.24	62.24
S9	1.00	19.72	19.72	1.00	58.22	58.22

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

Tooth	D11			D13			D23		
	Time point	Protein	HA	HA/ Protein	Protein	HA	HA/ Protein	Protein	HA
T0	N/A	N/A	N/A	6.00	88.51	14.75	2.69	87.01	32.34
L0	N/A	N/A	N/A	61.38	105.19	1.71	8.76	149.09	17.02
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	143.45	5.36	7.15	83.95	11.74	29.48	147.64	5.01
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	88.67	10.71	56.00	129.11	2.31	25.19	123.62	4.91
M1	25.97	168.13	6.47	61.00	155.56	2.55	11.26	96.29	8.55
M2	11.74	120.16	10.23	54.46	119.94	2.2	32.33	329.73	10.20
M3	7.51	50.09	6.67	36.77	124.35	3.38	10.19	58.05	5.70
M4xxx	10.97	377.78	34.42	28.69	158.77	5.53	44.83	196.66	4.39
M5	17.90	66.47	3.71	20.62	190.26	9.23	43.76	128.90	2.95
M6	9.05	98.45	10.88	61.77	118.44	1.92	17.33	80.40	4.64
M7	3.28	114.04	34.75	25.62	129.72	5.06	11.26	55.31	4.91
M8xxx	2.13	76.96	36.16	9.08	125.13	13.79	8.76	57.66	6.58
M9	1.00	28.68	28.68	1.38	33.39	24.11	4.12	56.60	13.74
M10	5.59	81.74	14.62	52.15	186.34	3.57	10.55	105.71	10.02
M11	10.59	133.46	12.6	36.77	94.47	2.57	9.12	68.74	7.54
M12	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
M13xxx	41.74	1045.70	25.05	133.31	229.26	1.72	81.26	386.54	4.76
M14	11.36	276.20	24.32	6.00	102.87	17.15	21.62	94.81	4.39
M15	34.44	289.12	8.4	21.38	60.29	2.82	55.90	105.00	1.88
M16	6.74	126.62	18.78	87.92	150.75	1.71	19.83	162.23	8.18
M17xxx	2.13	14.28	6.71	15.23	175.69	11.54	20.90	376.82	18.03
M18	2.90	30.29	10.45	4.46	117.68	26.38	21.98	1015.10	46.19
M19	0.97	107.47	110.3	2.54	135.45	53.36	13.05	790.51	60.59

Table A.2 (Continued)

Tooth	D11			D13			D23		
	Time point	Protein	HA/Protein	Protein	HA	HA/Protein	Protein	HA	HA/Protein
M20	2.13	140.71	66.12	29.85	131.94	4.42	18.76	253.29	13.50
M21xxx	5.59	364.73	65.25	18.31	228.12	12.46	35.19	1991.5	56.59
M22	1.00	25.94	25.94	26.77	236.14	8.82	11.98	262.83	21.95
M23	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
M24	1.00	13.80	13.8	8.69	205.16	23.6	9.48	208.54	22.01
M25xxx	2.90	120.44	41.57	3.69	231.51	62.7	33.40	176.06	5.27
M26	1.00	117.06	117.06	37.54	135.85	3.62	29.12	271.76	9.33
M27	1.95	76.74	39.38	12.15	163.16	13.42	64.83	1768.1	27.27
M28	1.00	73.22	73.22	1.77	55.37	31.29	29.48	918.81	31.17
M29	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
M30xxx	1.56	57.42	36.71	1.00	68.92	68.92	16.98	419.54	24.71
M31	3.10	110.08	35.48	11.77	157.88	13.41	37.33	289.66	7.76
M32	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
M33	2.72	98.58	36.27	1.00	75.82	75.82	0.55	116.97	213.60
M34xxx	3.87	16.42	4.24	19.46	188.83	9.7	63.76	1679.6	26.34
M35	8.87	59.14	6.67	1.00	117.68	117.68	85.19	1850.0	21.72
M36	12.33	189.03	15.33	36.77	168.14	4.57	13.40	287.01	21.41
M37	11.18	277.89	24.86	72.18	310.32	4.3	8.86	46.45	5.24
M38xxx	5.41	138.96	25.68	8.33	46.82	5.62	17.61	678.89	38.55
M39	18.49	252.81	13.67	58.33	833.75	14.29	53.03	765.32	14.43
S0	1.56	92.71	59.27	20.64	206.81	10.02	15.94	467.27	29.31
S1	4.26	145.97	34.29	54.49	112.20	2.06	36.36	834.12	22.94
S2	1.00	167.79	167.79	11.41	276.08	24.2	33.86	178.14	5.26
S3	6.56	165.64	25.23	52.95	621.49	11.74	31.36	1122.0	35.78
S4	20.79	231.65	11.14	24.87	290.16	11.67	13.03	33.89	2.60
S5	29.26	172.67	5.9	5.26	358.68	68.24	8.03	90.54	11.28
S6	15.79	349.82	22.15	12.56	197.41	15.71	1.00	17.06	17.06
S7	5.79	160.20	27.65	24.49	197.92	8.08	11.36	315.49	27.77
S8	9.64	163.10	16.92	20.26	657.18	32.44	1.00	11.55	11.55
S9	8.87	317.69	35.81	26.41	302.04	11.44	26.36	371.58	14.10

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

Tooth	E11			E13			E23		
	Protein	HA	HA/ Protein	Protein	HA	HA/ Protein	Protein	HA	HA/ Protein
T0	N/A	N/A	N/A	1.00	158.29	158.29	1.00	593.56	593.56
L0	19.69	1504.60	76.41	31.45	1537.30	48.87	1.00	1259.50	1259.50
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	42.08	11.89	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	18.67	15.17	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	5259.30	712.20	8.27	362.13	43.77	1.00	327.09	327.09
M1	3.54	200.40	56.63	12.36	1265.50	102.36	113.38	2123.40	18.73
M2	6.23	175.77	28.21	4.18	335.29	80.18	1.08	240.59	223.41
M3	2.38	18.45	7.74	1.00	143.73	143.73	1.00	244.53	244.53
M4xxx	47.00	1175.30	25.01	1.00	401.87	401.87	1.00	1369.10	1369.10
M5	5.46	148.53	27.20	15.09	1002.80	66.45	27.23	1042.00	38.27
M6	1.00	82.56	82.56	9.64	1570.40	162.97	14.92	2157.00	144.54
M7xxx	1.00	18.16	18.16	12.36	1815.10	146.81	7.62	2577.00	338.39
M8	17.77	665.79	37.47	1.00	256.88	256.88	26.85	1184.30	44.11
M9	1.00	83.78	83.78	24.64	1398.40	56.76	8.38	345.12	41.16
M10	6.62	212.34	32.08	8.73	813.31	93.19	8.38	1679.20	200.27
M11xxx	1.00	18.45	18.45	7.82	2367.70	302.85	17.62	1220.40	69.28
M12	N/A	N/A	N/A	33.73	73.22	2.17	30.69	152.96	4.98
M13	46.23	102.11	2.21	34.64	580.80	16.77	45.31	1557.00	34.37
M14	1.00	6.17	6.17	9.18	1830.50	199.36	30.69	2298.80	74.90

Table A.3 (Continued)

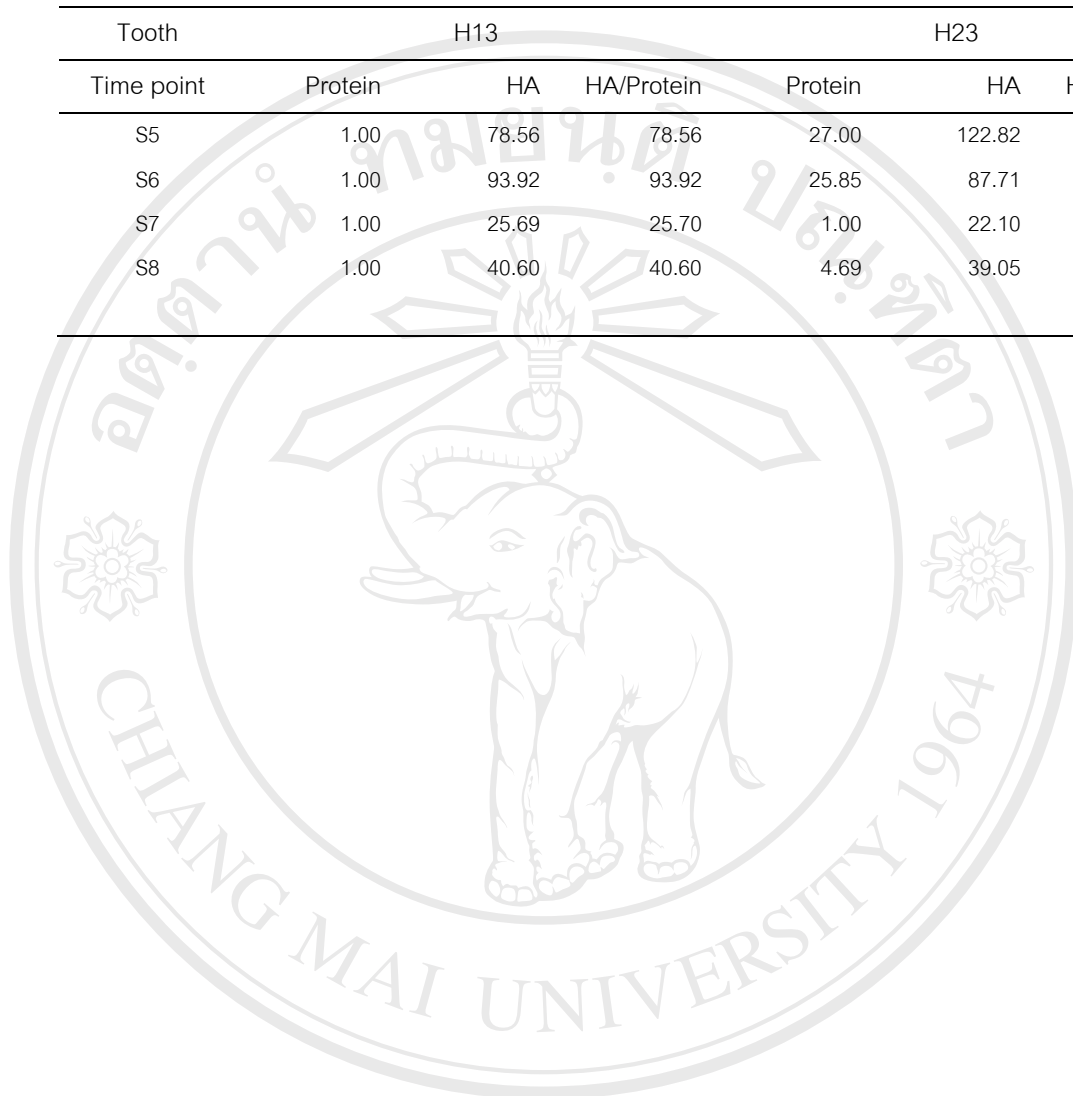
Tooth	E11			E13			E23		
	Time point	Protein	HA/Protein	Protein	HA	HA/Protein	Protein	HA	HA/Protein
M15xxx	1.00	82.24	82.24	1.00	164.98	164.98	11.46	773.29	67.47
M16	1.00	32.51	32.51	7.36	223.97	30.42	4.92	332.00	67.44
M17	7.38	216.85	29.37	8.73	830.03	95.11	18.38	92.47	5.03
M18	1.00	33.44	33.44	6.91	239.12	34.61	12.23	37.19	3.04
M19xxx	1.00	53.85	53.85	1.00	34.67	34.67	21.08	1308.10	62.06
M20	23.54	13.41	0.57	1.00	24.08	24.08	6.08	1173.40	193.09
M21	5.46	30.31	5.55	10.55	385.81	36.59	6.08	226.38	37.25
M22	2.77	59.09	21.34	6.00	41.83	6.97	1.00	81.18	81.18
M23xxx	1.00	20.15	20.15	3.73	97.81	26.24	6.08	1320.70	217.33
M24	1.00	24.06	24.06	69.64	96.50	1.39	101.46	97.36	0.96
M25	8.92	99.43	11.14	46.00	195.55	4.25	N/A	N/A	N/A
M26	9.69	N/A	0.00	4.18	30.87	7.38	N/A	N/A	N/A
M27xxx	7.38	37.25	5.04	62.82	113.57	1.81	N/A	N/A	N/A
S0	1.00	18.60	18.60	1.00	36.71	36.71	13.38	1691.50	126.38
S1	1.00	40.95	40.95	6.45	230.37	35.69	9.54	443.51	46.50
S2	N/A	N/A	N/A	4.64	207.39	44.73	31.08	620.07	19.95
S3	N/A	N/A	N/A	17.82	40.83	2.29	2.23	28.74	12.88
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	94.79	10.86	18.38	2977.40	161.95
S6	N/A	N/A	N/A	53.73	877.48	16.33	23.77	2326.20	97.87
S7	N/A	N/A	N/A	1.00	127.89	127.89	31.08	601.14	19.34
S8	1.00	N/A	N/A	1.00	881.52	881.52	3.77	5.39	1.43
S9	N/A	N/A	N/A	N/A	N/A	N/A	21.85	541.29	24.78

Table A.4 Protein levels, HA levels, HA levels per unit protein of GCF samples from H13, H23 (N/A = not available, xxx means the time point when an orthodontic force was applied to the canine.)

Tooth	H13			H23			
	Time point	Protein	HA	HA/Protein	Protein	HA	HA/Protein
T0		1.00	82.95	82.95	2.00	177.72	88.86
L0		35.51	564.56	15.90	80.85	525.53	6.50
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		13.70	214.07	15.64	33.92	87.29	2.57
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
M0xxx		15.30	388.10	25.37	14.31	333.71	23.32
M1		11.73	126.10	10.75	7.38	115.03	15.58
M2		6.40	463.97	72.44	63.54	354.48	5.58
M3		4.80	181.85	37.90	45.08	340.56	7.56
M4xxx		92.30	179.63	1.95	15.46	143.61	9.29
M5		11.20	211.77	18.92	8.92	113.78	12.75
M6		1.00	79.75	79.75	1.00	28.44	28.44
M7		1.00	31.41	31.41	1.00	56.86	56.86
M8xxx		12.80	38.02	2.97	N/A	N/A	N/A
M9		1.00	24.83	24.83	1.00	28.55	28.55
M10		N/A	N/A	N/A	N/A	N/A	N/A
M11		1.00	32.72	32.72	1.00	29.78	29.78
M12xxx		8.01	248.48	31.01	5.08	80.36	15.83
M13		1.00	68.78	68.78	1.00	12.70	12.70
M14		1.00	53.70	53.70	1.00	18.74	18.74
M15		1.00	41.53	41.53	1.00	31.33	31.33
S0		1.00	16.42	16.42	1.00	42.09	42.09
S1		1.00	33.98	33.98	1.00	4.44	4.44
S2		1.00	34.79	34.79	0.46	16.64	36.05
S3		1.00	90.09	90.09	29.31	237.18	8.09
S4		2.64	109.00	41.24	51.62	96.55	1.87

Table A.4 (Continued)

Tooth	H13			H23		
	Protein	HA	HA/Protein	Protein	HA	HA/Protein
S5	1.00	78.56	78.56	27.00	122.82	4.55
S6	1.00	93.92	93.92	25.85	87.71	3.39
S7	1.00	25.69	25.70	1.00	22.10	22.10
S8	1.00	40.60	40.60	4.69	39.05	8.32



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