

APPENDIX B

Calcium Imaging Studies						
Rabbit	Sample	Shear Stress (Pa)	Fluid Flow Results		Baseline Response	
			% Cell Response	340:380 Amp Ratio	% Cell Response	340:380 Amp Ratio
6	1	1.5	46.4	1.45	0.0	0.00
	2	1.5	27.3	4.76	9.1	1.90
	3	2.5	27.3	1.68	0.0	0.00
	4	2.5	17.7	1.59	0.0	0.00
	5	4	31.3	1.72	0.0	0.00
	6	4	58.6	2.12	3.5	1.66
	7	5	50.0	1.07	0.0	0.00
	8	5	46.2	1.09	3.8	0.65
	9	6.5	88.5	4.31	7.7	2.90
	10	6.5	64.5	3.31	9.4	2.05
7	1	1.5	8.0	1.25	0.0	0.00
	2	2.5	20.0	2.21	0.0	0.00
	3	4	15.4	1.00	0.0	0.00
	4	4	66.7	3.32	5.6	2.60
	5	5	41.4	1.62	0.0	0.00
	6	5	54.5	0.47	0.0	0.00
	7	6.5	25.0	1.26	3.4	0.93
	8	6.5	86.7	1.44	0.0	0.00
8	1	1.5	21.1	1.01	0.0	0.00
	2	2.5	74.1	1.17	0.0	0.00
	3	4	72.4	1.82	0.0	0.00
	4	5	59.1	5.30	0.0	0.00
	5	6.5	74.1	1.73	0.0	0.00
10	1	1.5	33.3	1.25	0.0	0.00
	2	2.5	86.5	2.08	5.1	1.01
	3	4	88.0	4.59	4.0	1.98
	4	5	65.2	2.21	8.7	2.39
	5	6.5	96.4	1.62	0.0	0.00
11	1	1.5	20.8	4.86	0.0	0.00
	2	2.5	81.3	1.56	3.1	0.76
	3	4	80.0	1.07	0.0	0.00
	4	5	94.6	1.76	0.0	0.00
	5	5	84.0	2.57	0.0	0.00
	6	6.5	56.0	2.50	8.0	2.36

Table 1.1. Summary of the $[Ca^{2+}]_i$ response of meniscal cells to OFF induced shear stress with the application of fluid flow at various shear stress levels, and no flow control baseline level.

Calcium Imaging Studies with Calcium Blocked by Thapsigargin				
Rabbit	Sample	Shear Stress (Pa)	Fluid Flow Results	
			% Cell Response	340:380 Amp Ratio
F	1	5	0.0	0.00
	2	5	0.0	0.00
	3	5	0.0	0.00
	4	5	0.0	0.00
	5	5	6.3	1.23
13	1	4	3.3	1.11
	2	4	5.6	1.14
	3	4	2.9	3.10
	4	4	0.0	0.00
14	1	4	21.1	1.01
	2	4	74.4	3.85
	3	4	2.2	1.10
	4	4	59.1	5.30

Table 1.2. Summary of the $[Ca^{2+}]_i$ response of meniscal cells to OFF induced shear stress at various shear stress levels with $[Ca^{2+}]_i$ blocked by the pharmacological agent thapsigargin.

Rabbit	Sample	Experimental Condition & Incubation Period					
		4 pa 1 day			No Flow 1 day		
		Total GAG μg	Total Protein μg	Normalized (ng GAG/ μg TP)	Total GAG μg	Total Protein μg	Normalized (ng GAG/ μg TP)
10 IBV: 1ml	1	12.36	357.93	34.54	15.79	80.69	195.63
	2	15.50	105.17	147.34	18.34	95.17	192.70
Avg 10		13.93	231.55	90.94	17.06	87.93	194.16
11 IBV: 1ml	1	29.72	325.00	91.43		135.44	0.00
	2	53.41	174.68	305.75	0.00	166.46	0.00
Avg 11		41.56	249.84	198.59	0.00	150.95	0.00
12 CL: 100 μl	1	6.71	42.05	84.11			
	2	10.79	40.82	81.64			
	3	10.87	35.10	70.21			
	4	11.66	33.66	67.33			
Avg. 12		10.01	37.91	75.82			

Table 2.1. Summary of the Protein Production Studies for 1 day incubations for OFF induced shear stress of 4 Pa and the no flow condition. Includes measures of Total Sulfated GAG (μg) by the modified DMMB method, Total Cell Protein (μg) by the Lowry Method, and the Normalized Total Sulfated GAG (ng)/Total Cell Lysate Protein (μg). Total Sulfated GAG and Total Cell Protein values have already been multiplied by their respective culture media volumes (8.75 ml) and isolation buffer volumes (IBV).

Rabbit	Sample	Experimental Condition & Incubation Time					
		4 Pa 2 day			No Flow 2 day		
		Total GAG μg	Total Protein μg	Normalized (ng GAG/ μg TP)	Total GAG μg	Total Protein μg	Normalized (ng GAG/ μg TP)
10 IBV: 1ml	1	27.73	215.52	128.67	21.91	201.03	108.96
	2	56.91	143.79	395.76	22.17	179.31	123.66
Avg 10		42.32	179.66	262.22	22.04	190.17	116.31
11 IBV: 1ml	1	11.28	146.20	77.14	12.70	181.65	69.91
	2	9.51	143.67	66.22		236.08	0.00
Avg 11		10.40	144.94	71.68	6.35	208.86	34.96
12 CL: 100 μl	1	11.03	86.34	127.79			
	2	18.55	93.97	197.39			
	3	8.13	97.23	83.57			
	4	16.19	75.45	214.53			
Avg 12		13.47	88.25	155.82			

Table 2.2. Summary of the Protein Production Studies for 2 day incubations for OFF induced shear stress of 4 Pa and the no flow condition. Includes measures of Total Sulfated GAG (μg) by the modified DMMB method, Total Cell Protein (μg) by the Lowry Method, and the Normalized Total Sulfated GAG (ng)/Total Cell Lysate Protein (μg). Total Sulfated GAG and Total Cell Protein values have already been multiplied by their respective culture media volumes (8.125 ml) and isolation buffer volumes (IBV).

Rabbit	Sample	Experimental Condition & Incubation Time					
		4 Pa 3 day			No Flow 3 day		
		Total GAG µg	Total Protein µg	Normalized (ng GAG/µg TP)	Total GAG µg	Total Protein µg	Normalized (ng GAG/µg TP)
10 IBV: 1 ml	1	51.97	113.45	458.07	36.35	422.07	86.11
	2	27.35	144.48	189.26	36.78	193.79	189.79
Avg. 10				323.67			137.95
11 IBV: 1 ml	1	15.78	191.46	82.42	12.72	383.86	33.14
	2	50.35	114.87	438.29	17.78	196.84	90.34
Avg 11				260.35			61.74
12 CL: 100µl	1	18.38	72.02	255.14			
	2	53.63	95.89	559.23			
	3	25.19	80.72	312.01			
	4	33.50	83.01	403.58			
Avg. 12			382.49				
13 IBV: 0.5 ml	1	51.75	106.94	483.90	25.56	112.50	227.20
	2	19.07	100.87	189.01	27.69	103.13	268.51
	3	15.81	122.92	128.62	21.75	54.86	396.46
Avg. 13			267.18			297.39	
14 IBV: 0.5 ml	1	14.75	51.91	284.20	5.94	73.61	80.69
	2	24.06	63.89	376.59	2.57	123.26	20.81
Avg. 14				330.39			50.75
15 IBV: 0.5 ml	1	16.47	227.44	72.42	26.72	114.12	234.09
	2	25.40	88.80	286.07	42.10	172.40	244.18
	3	24.78	68.51	361.72			
Avg. 15				240.07			239.14
16 IBV: 0.5 ml	1	25.28	149.51	169.10	56.22	152.44	368.81
	2	42.03	193.83	216.84	56.97	144.64	393.87
	3	44.60	154.71	288.25	33.03	136.36	242.22
	4	43.06	285.48	150.84	20.03	326.47	61.37
	5	74.76	349.08	214.16	21.68	221.51	97.88
Avg. 16				203.62			207.30
17 IBV: 0.5 ml	1	34.66	175.65	197.31	39.41	158.12	249.21
	2	32.16	154.06	208.75	37.04	172.24	215.02
	3	53.84	135.71	396.73	88.10	188.47	467.41
	4	27.26	283.64	96.09	22.80	365.26	62.43
	5	62.46	323.53	193.05	23.89	300.22	79.57
	6	30.11	264.34	113.89			
	7	63.43	231.80	273.65			
Avg. 17				218.39			190.78
18 IBV: 0.5 ml	1				32.84	210.48	156.04
	2				53.90	179.23	300.71
Avg. 18							228.37
19 IBV: 0.5 ml	1				27.34	503.68	54.28
	2				32.79	606.25	54.10

					18.80	177.76	105.78
					17.30	169.49	102.09
Avg. 19							83.56
20 IBV: 0.5 ml	1				17.15	270.77	63.32
	2				17.03	181.99	93.59
	3	23.91	321.51	74.35	30.33	429.04	70.69
	4	12.36	435.66	28.36	34.34	318.46	107.82
Avg. 20				51.36		83.86	

Table 2.3 Summary of the Protein Production Studies for 3 day incubations for OFF induced shear stress of 4 Pa and the no flow condition. Includes measures of Total Sulfated GAG (μg) by the modified DMMB method, Total Cell Protein (μg) by the Lowry Method, and the Normalized Total Sulfated GAG (ng)/Total Cell Lysate Protein (μg). Total Sulfated GAG and Total Cell Protein values have already been multiplied by their respective culture media volumes (7.5 ml) and isolation buffer volumes (IBV).

Rabbit	Sample	Experimental Condition & Incubation Time					
		1.5 Pa 3 day			No Flow 3 day		
		Total GAG μg	Total Protein μg	Normalized (ng GAG/μg TP)	Total GAG μg	Total Protein μg	Normalized (ng GAG/μg TP)
18	1	12.65	138.79	91.11	32.84	210.48	156.04
IBV: 0.5 ml	2	11.90	62.13	191.45	53.90	179.23	300.71
Avg. 18				141.28			228.37
19	1	12.16	63.05	192.82	27.34	503.68	54.28
IBV: 0.5 ml	2	39.11	98.35	397.63	32.79	606.25	54.10
Avg. 19				295.22	18.80	177.76	105.78
20	1	21.65	119.49	181.15	17.30	169.49	102.09
IBV: 0.5 ml	2	13.77	106.25	129.60			83.56
Avg. 20				155.38	17.15	270.77	63.32

Table 2.4. Summary of the Protein Production Studies for 3 day incubations for OFF induced shear stress of 1.5 Pa. Includes measures of Total Sulfated GAG (μg) by the modified DMMB method, Total Cell Protein (μg) by the Lowry Method, and the Normalized Total Sulfated GAG (ng)/Total Cell Lysate Protein (μg). Total Sulfated GAG and Total Cell Protein values have already been multiplied by their respective culture media volumes (7.5 ml) and isolation buffer volumes (IBV).

Rabbit	Sample	Experimental Condition & Incubation Time					
		Ca Block 3 day 4 Pa-1hr Flow			Ca Block 3 day No Flow-1hr		
		Total GAG μg	Total Protein μg	Normalized (ng GAG/ μg TP)	Total GAG μg	Total Protein μg	Normalized (ng GAG/ μg TP)
13 IBV: 0.5 ml	1	29.69	239.41	123.99			
	2	56.38	89.76	628.11			
	3	25.69	130.38	197.02			
	4	23.81	139.24	171.02			
	5	13.00	128.65	101.03			
	6	17.75	223.61	79.39			
Avg. 13				216.76			
14 IBV: 0.5 ml	1	8.06	217.19	37.12			
	2	11.69	190.28	61.41			
	3	5.38	153.99	34.92			
	4	5.75	129.17	44.54			
	5	10.88	168.58	64.51			
	6	45.13	107.99	417.90			
Avg. 14				110.07			
15 IBV: 0.5 ml	1	50.28	166.23	302.47	38.35	153.90	249.18
	2	31.97	153.41	208.41	37.04	329.22	112.49
	4	36.10	137.62	262.30	40.41	127.60	316.70
	5	63.53	146.43	433.88	27.53	218.67	125.91
	6	32.47	234.09	138.70			
	Avg. 15			269.15			201.07
16 IBV: 0.5 ml	1	27.97	180.52	154.93			
	2	17.97	256.49	70.06			
	3	30.97	175.81	176.14			
	4	26.04	456.62	57.02	26.76	275.18	97.25
	5	25.71	339.71	75.69	27.27	345.59	78.91
	Avg. 16			100.04			88.08
17 IBV: 0.5 ml	1	29.54	282.47	104.56	23.91	159.25	150.14
	2	30.72	133.28	230.49	24.91	204.38	121.87
	3	18.47	145.29	127.14	35.97	140.75	255.56
Avg. 17			154.07			175.86	

Table 2.5. Summary of the Protein Production Studies for 3 day incubations for OFF induced shear stress of 4 Pa and the no flow condition with $[\text{Ca}^{2+}]_i$ blocked by the pharmacological agent thapsigargin. Includes measures of Total Sulfated GAG (μg) by the modified DMMB method, Total Cell Protein (μg) by the Lowry Method, and the Normalized Total Sulfated GAG (ng)/Total Cell Lysate Protein (μg). Total Sulfated GAG and Total Cell Protein values have already been multiplied by their respective culture media volumes (7.5 ml) and isolation buffer volumes (IBV).

Rabbit Specimen	Mayo Lab Group Received From	OFF Duration (hr)
6	Dr. O'Driscoll	-----
7	Dr. O'Driscoll	-----
8	Dr. O'Driscoll	-----
10	Dr. O'Driscoll	-----
11	Dr. O'Driscoll	2
12	Dr. O'Driscoll	2
13	Dr. Lewis	1.5
14	Dr. Lewis	1
15	Dr. Lewis	1
16	Dr. Lewis	1
17	Dr. Lewis	1
18	Dr. Lewis	1
19	Dr. Lewis	1
20	Dr. Lewis	1

Table 3. Documentation of Mayo Clinic lab group which rabbit specimens were received, and the OFF duration that each specimen was exposed to during protein studies. Rabbit legs received from the lab of Dr. Lewis were larger than those received from Dr. O'Driscoll's. Specimen use was also different between groups, neither of which affected the meniscus in adverse ways.