

Table 1 Clinical signs in rabbits

Group	Animal No.	Days after placement																													
		0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29
Control	M01101	N	A	A	N	N	N	N	N	Necropsied 7 days after placement																					
	M01102	N	N	N	N	N	N	N	N	Necropsied 7 days after placement																					
	M01103	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
	M01104	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
	Number of animals	4	4	4	4	4	4	4	4	4	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2
	N	4	3	3	4	4	4	4	4	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2
A	0	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	
gMSC	M02201	N	A	N	N	N	N	N	N	Necropsied 7 days after placement																					
	M02202	N	A	A	A	A	A	N	N	Necropsied 7 days after placement																					
	M02203	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
	M02204	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
	Number of animals	4	4	4	4	4	4	4	4	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2
	N	4	2	3	3	3	3	4	4	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2
A	0	2	1	1	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	
Dil-gMSC	M03301	N	A	N	N	N	N	N	N	Necropsied 7 days after placement																					
	M03302	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
	Number of animals	2	2	2	2	2	2	2	2	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	
	N	2	1	2	2	2	2	2	2	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	
	A	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

N: Normal.

A: Decrease in locomotor activity.

Table 2 Body weights of rabbits

Group	Animal No.	Days after placement					
		0	7	14	21	28	29
Control	M01101	1.93	1.91	Necropsied 7 days after placement			
	M01102	1.79	1.89	Necropsied 7 days after placement			
	M01103	1.71	1.84	1.98	2.14	2.31	2.32
	M01104	1.97	2.10	2.24	2.37	2.49	2.51
	Number of animals	4	4	2	2	2	2
	Mean	1.85	1.94	2.11	2.26	2.40	2.42
	gMSC	M02201	1.83	1.87	Necropsied 7 days after placement		
M02202		1.74	1.56	Necropsied 7 days after placement			
M02203		1.79	1.84	2.05	2.23	2.35	2.36
M02204		1.96	2.11	2.33	2.46	2.62	2.68
Number of animals		4	4	2	2	2	2
Mean		1.83	1.85	2.19	2.35	2.49	2.52
Dil-gMSC		M03301	1.77	1.87	Necropsied 7 days after placement		
	M03302	1.84	1.83	2.07	2.35	2.49	2.48
	Number of animals	2	2	1	1	1	1
	Mean	1.81	1.85	2.07	2.35	2.49	2.48
	Unit: kg						

Table 3 Food consumption in rabbits

Group	Animal No.	Days after placement															
		0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
Control	M01101	130	10	2	50	109	119	130	130	Necropsied 7 days after placement							
	M01102	130	50	111	116	130	130	130	130	Necropsied 7 days after placement							
	M01103	130	61	57	107	120	112	130	130	130	130	130	130	130	130	130	130
	M01104	130	71	130	130	130	130	130	130	130	130	130	130	130	130	130	130
	Number of animals	4	4	4	4	4	4	4	4	4	2	2	2	2	2	2	2
	Mean	130	48	75	101	122	123	130	130	130	130	130	130	130	130	130	130
gMSC	M02201	113	16	87	123	121	114	109	115	Necropsied 7 days after placement							
	M02202	105	0	0	9	2	20	89	113	Necropsied 7 days after placement							
	M02203	121	42	92	111	110	119	106	112	118	121	124	130	130	130	130	
	M02204	130	119	130	130	130	130	130	130	130	130	130	130	130	130	130	130
	Number of animals	4	4	4	4	4	4	4	4	4	2	2	2	2	2	2	2
	Mean	117	44	77	93	91	96	109	118	124	126	127	130	130	130	130	130
Dil-gMSC	M03301	130	16	95	126	130	130	130	130	Necropsied 7 days after placement							
	M03302	130	102	130	130	130	130	130	130	130	130	130	130	130	130	130	
	Number of animals	2	2	2	2	2	2	2	2	2	1	1	1	1	1	1	1
	Mean	130	59	113	128	130	130	130	130	130	130	130	130	130	130	130	130

Unit: g/day.

(Continued)

Table 3 (Continued) Food consumption in rabbits

Group	Animal No.	Days after placement													
		16	17	18	19	20	21	22	23	24	25	26	27	28	29
Control	M01101	Necropsied 7 days after placement													
	M01102	Necropsied 7 days after placement													
	M01103	130	130	130	130	130	130	130	130	130	130	130	130	130	130
	M01104	130	130	130	130	130	130	130	130	130	130	130	130	130	130
	Number of animals	2	2	2	2	2	2	2	2	2	2	2	2	2	2
	Mean	130	130	130	130	130	130	130	130	130	130	130	130	130	130
gMSC	M02201	Necropsied 7 days after placement													
	M02202	Necropsied 7 days after placement													
	M02203	130	130	130	130	130	130	130	130	130	130	130	130	130	130
	M02204	130	130	130	130	130	130	130	130	130	130	130	130	130	130
	Number of animals	2	2	2	2	2	2	2	2	2	2	2	2	2	2
	Mean	130	130	130	130	130	130	130	130	130	130	130	130	130	130
Dil-gMSC	M03301	Necropsied 7 days after placement													
	M03302	130	130	130	130	130	130	130	130	130	130	130	130	130	130
	Number of animals	1	1	1	1	1	1	1	1	1	1	1	1	1	1
	Mean	130	130	130	130	130	130	130	130	130	130	130	130	130	130

Unit: g/day.

Table 4 Necropsy findings in rabbits

Group	Animal No.	Findings	
Control	M01101	All organs and tissues	Normal
	M01102	All organs and tissues	Normal
	M01103	All organs and tissues	Normal
	M01104	All organs and tissues	Normal
gMSC	M02201	All organs and tissues	Normal
	M02202	All organs and tissues	Normal
	M02203	All organs and tissues	Normal
	M02204	All organs and tissues	Normal
Dil-gMSC	M03301	All organs and tissues	Normal
	M03302	All organs and tissues	Normal

Table 5-1 Histopathological findings in rabbits 7 days after placement

Control group		
Animal No.	Organ/Tissue	Finding
M01101	Left knee joint	Proliferation, synovial membrane: ± Infiltrate, inflammatory cell, synovial membrane: ± Free body (amorphous basophilic body): ±
	Right knee joint	Proliferation, synovial membrane: ± Infiltrate, inflammatory cell, synovial membrane: ± Free body (amorphous basophilic body): ±
M01102	Left knee joint	Proliferation, synovial membrane: ± Infiltrate, inflammatory cell, synovial membrane: ± Free body (amorphous basophilic body): ±
	Right knee joint	Proliferation, synovial membrane: ± Infiltrate, inflammatory cell, synovial membrane: ± Free body (amorphous basophilic body): ±

Grade of histopathological findings: ±: slight, +: mild, 2+: moderate, 3+: marked.

Table 5-2 Histopathological findings in rabbits 7 days after placement

gMSC group		
Animal No.	Organ/Tissue	Finding
M02201	Left knee joint	Proliferation, synovial membrane: + Infiltrate, inflammatory cell, synovial membrane (lymphocytic): + Infiltrate, inflammatory cell, synovial fluid: ± Free body (cellular eosinophilic body): +
	Right knee joint	Proliferation, synovial membrane: + Infiltrate, inflammatory cell, synovial membrane (lymphocytic): + Infiltrate, inflammatory cell, synovial fluid: + Free body (cellular eosinophilic body): +
M02202	Left knee joint	Proliferation, synovial membrane: + Infiltrate, inflammatory cell, synovial membrane (lymphocytic): 2+ Free body (cellular eosinophilic body): +
	Right knee joint	Proliferation, synovial membrane: + Infiltrate, inflammatory cell, synovial membrane (lymphocytic): + Infiltrate, inflammatory cell, synovial fluid: + Free body (cellular eosinophilic body): +

Grade of histopathological findings: ±: slight, +: mild, 2+: moderate, 3+: marked.

Table 5-3 Histopathological findings in rabbits 29 days after placement

Control group		
Animal No.	Organ/Tissue	Finding
M01103	Left knee joint	Proliferation, synovial membrane: ± Infiltrate, inflammatory cell, synovial membrane: ± Free body (amorphous basophilic body): ±
	Right knee joint	Proliferation, synovial membrane: ± Infiltrate, inflammatory cell, synovial membrane: ± Free body (amorphous basophilic body): ±
M01104	Left knee joint	Proliferation, synovial membrane: ± Infiltrate, inflammatory cell, synovial membrane (granulocytic): + Free body (amorphous basophilic body): ±
	Right knee joint	Proliferation, synovial membrane: + Infiltrate, inflammatory cell, synovial membrane (granulocytic): + Free body (amorphous basophilic body): ±

Grade of histopathological findings: ±: slight, +: mild, 2+: moderate, 3+: marked.

Table 5-4 Histopathological findings in rabbits 29 days after placement

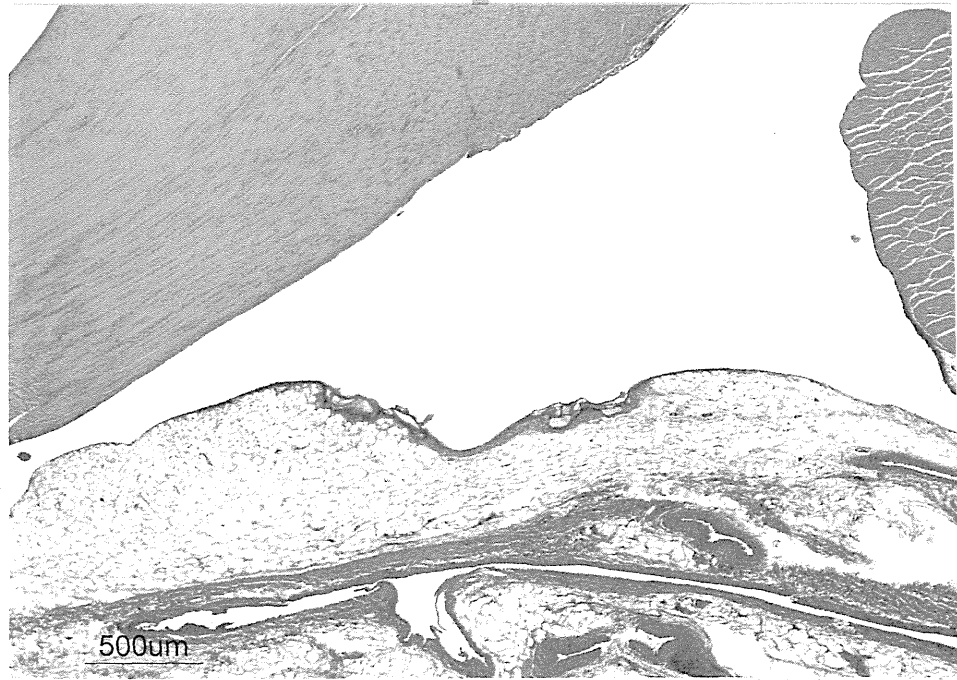
gMSC group		
Animal No.	Organ/Tissue	Finding
M02203	Left knee joint	Proliferation, synovial membrane: ± Infiltrate, inflammatory cell, synovial membrane (lymphocytic): + Free body (cellular eosinophilic body): ±
	Right knee joint	Proliferation, synovial membrane: + Infiltrate, inflammatory cell, synovial membrane (lymphocytic): + Infiltrate, inflammatory cell, synovial fluid: ± Free body (cellular eosinophilic body): +
M02204	Left knee joint	Proliferation, synovial membrane: + Infiltrate, inflammatory cell, synovial membrane (lymphocytic): + Free body (cellular eosinophilic body): +
	Right knee joint	Proliferation, synovial membrane: + Infiltrate, inflammatory cell, synovial membrane (lymphocytic): + Infiltrate, inflammatory cell, synovial fluid: ± Free body (cellular eosinophilic body): +

Grade of histopathological findings: ±: slight, +: mild, 2+: moderate, 3+: marked.

No.

Photo 1
Animal No. M01101
Left knee joint

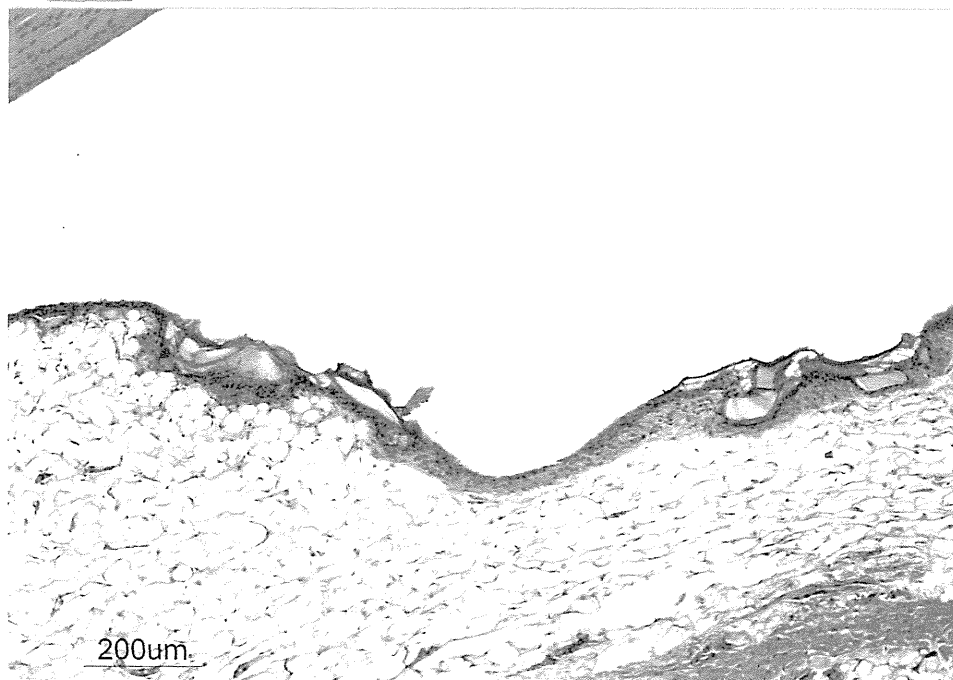
HE stain



No.

Photo 2
Animal No. M01101
Left knee joint

HE stain

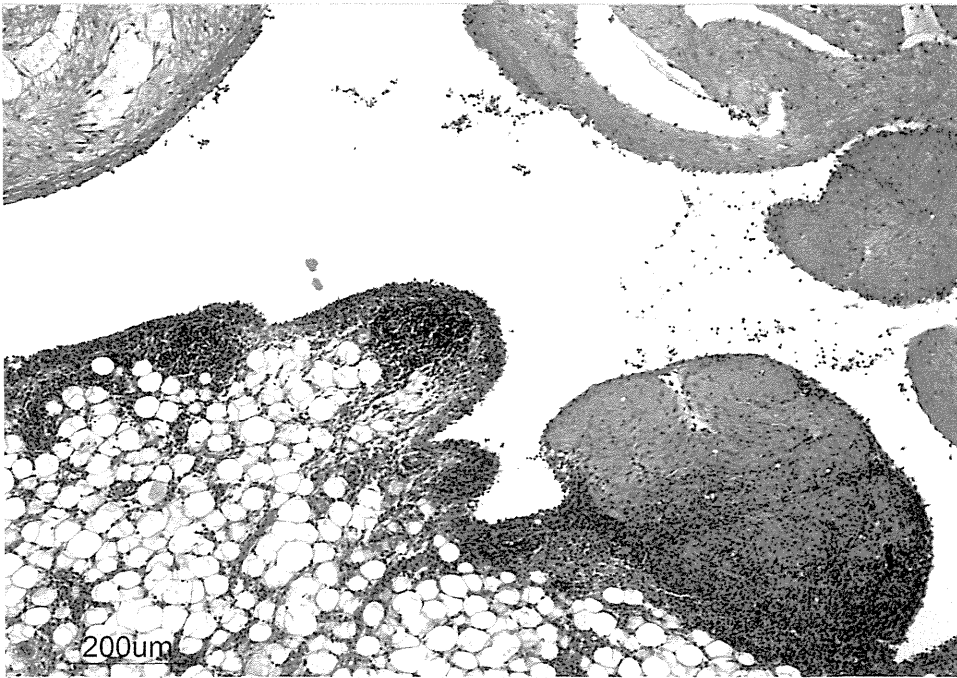


No.

Photo 3
Animal No. M02201
Left knee joint

HE stain

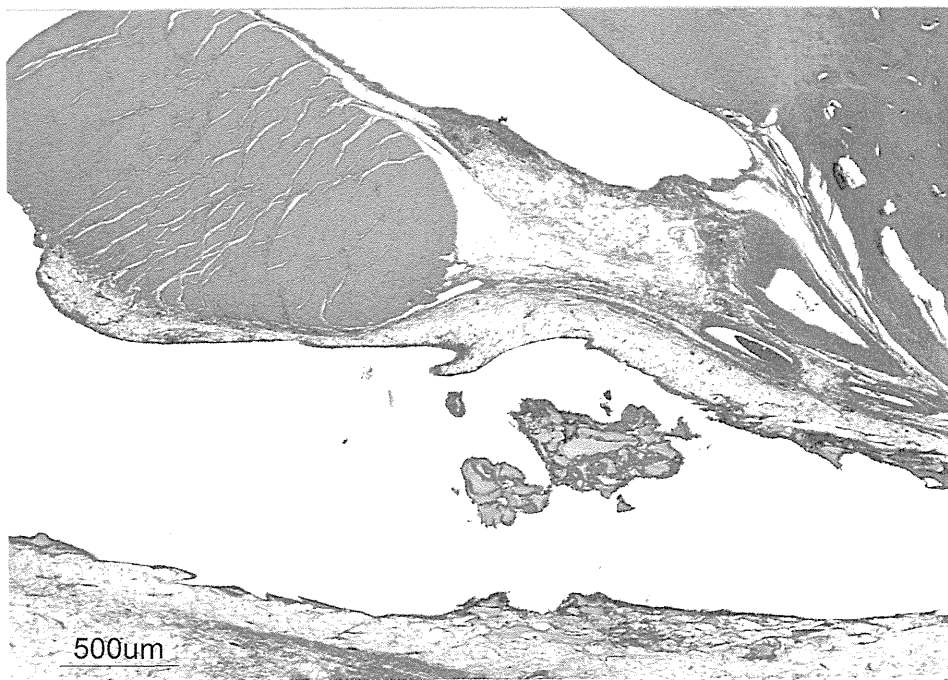




No.

Photo 4
Animal No. M02201
Left knee joint

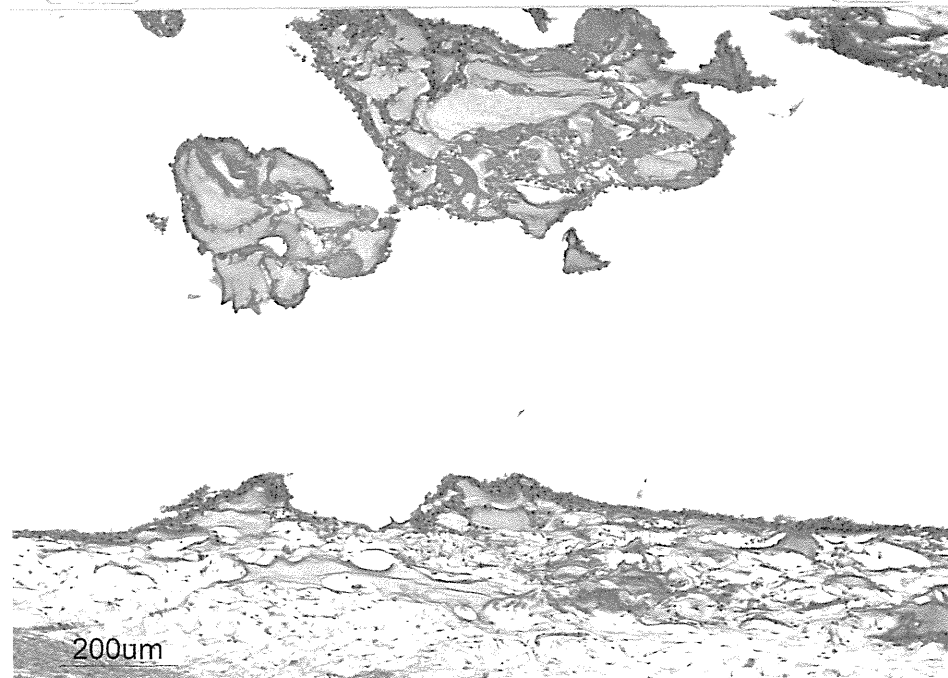
HE stain



No.

Photo 5
Animal No. M01103
Left knee joint

HE stain



No.

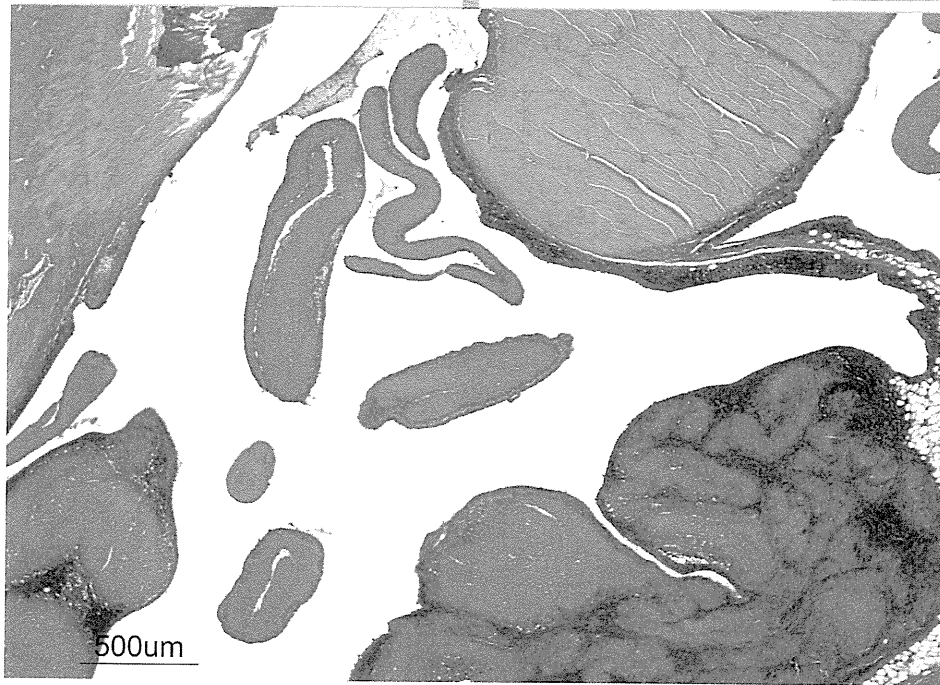
Photo 6
Animal No. M01103
Left knee joint

HE stain

No. _____

Photo 7
Animal No. M02203
Right knee joint

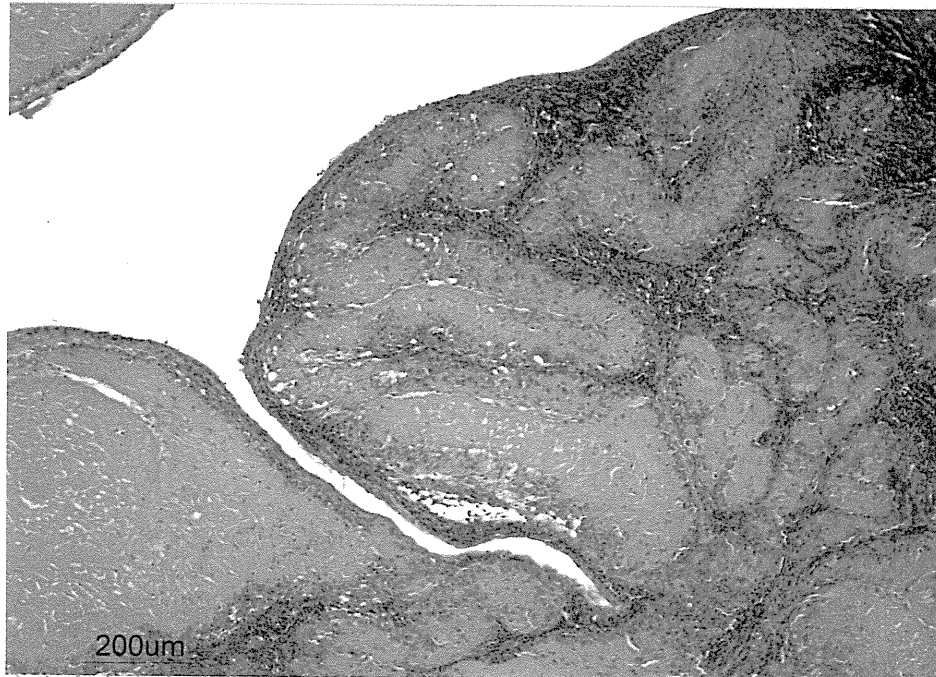
HE stain



No. _____

Photo 8
Animal No. M02203
Right knee joint

HE stain



No. _____

E.L.♦

Attachment 1-1

輸送後の gMSC 細胞生存率測定方法

Attachment 1-2

1. 作業手順

1.1 使用試薬、機器、器具又は容器の確認

1.1.1 品質管理検体及び使用試薬

名称	備考
製品名：品質管理検体 gMSC メーカー：ツーセル 管理番号：生存率 (1), (2), (9)	管理番号を記載 50 mL チューブに20 mL の輸送液を含有
製品名：0.2%コラゲナーゼ溶液 (20 mL) メーカー：ツーセル (社内調製) 管理番号：1025-(1), (2), (9)	ボトル毎で管理番号を記載 DMEM でコラゲナーゼを溶解させたもの
製品名：トリパンブルー溶液 0.4% (分注) メーカー：ツーセル (社内調製) 管理番号：RNBC2656	ボトル毎で管理番号を記載

1.1.2 使用機器

名称	備考
製品名：CO ₂ インキュベーター (BNA-121D) メーカー：エスベック株式会社 ID 番号：N-8、N-9	
製品名：多本架冷却遠心機 (CF5RX) メーカー：日立工機株式会社 ID 番号：M-7	
製品名：倒立型リサーチ顕微鏡 (CKX41) メーカー：オリンパス株式会社 ID 番号：K-37	
製品名：ボルテックス (S-50) メーカー：大洋科学工業株式会社 ID 番号：C-3	

1.1.3 使用器具又は容器

名称	備考
製品名：ディスポイン メーカー：アズワン 型番：0-2690-04	
製品名：ワンセルカウンター (血球計算盤) メーカー：BMS 型番：BMS-OCC01	
製品名：20 µl チップ メーカー：日本ジェネティクス 型番：14211	
製品名：セイフロックチューブ バイオピュア 0.5 mL メーカー：Eppendorf 型番：95191	
製品名：mLINE 手動ピペット (2.0-20 µl) メーカー：ザルトリウス 型番：725030	

Attachment 1-3

1.2 <生存率測定>

以下の手順に従って、安全キャビネット内で gMSC の生存率測定を行う。

- (1) 実験開始時間を記載後、安全キャビネット内で、梱包されている品質管理用 gMSC を開封し、ピンセットを用いて50 mL チューブ（0.2%コラゲナーゼ溶液20 mL 含有）の中に移し入れる。
- (2) CO₂インキュベーターを用いて37°C で90分間、時折混和（30分おきボルテックス等で攪拌）しながら加温する。
- (3) コラゲナーゼ反応中に mLINe 手動ピペット（2.0-20 μl）を用いて、セイフロックチューブにトリパンプルー溶液を10 μL ずつ必要数、分注しておく。
- (4) 90分後、ボルテックスで50 mL チューブを混和させ、gMSC が完全に分解されたことを確認する。
- (5) mLINe 手動ピペット（2.0-20 μl）を用いて、50 mL チューブより滑膜幹細胞懸濁液を10 μL 採取し、「(3)」で用意したセイフロックチューブ内のトリパンプルー溶液と混合させ、血球計算盤を用いて4区画中の生細胞、死細胞を数え、細胞懸濁液の細胞濃度を算出する。
- (6) 3回測定を行うために、ボルテックスで適宜攪拌を行い、あと2回測定を行う。

各種数値の算出方法は以下の式を用いる。

【ヒト滑膜幹細胞の生細胞濃度】

$$\left(\text{左上区画} + \text{右上区画} + \text{右下区画} + \text{左下区画} \right) \div 4 \times 2 \times 10^4 = \boxed{(1)} \text{ (cells/mL)}$$

【ヒト滑膜幹細胞の死細胞濃度】

$$\left(\text{左上区画} + \text{右上区画} + \text{右下区画} + \text{左下区画} \right) \div 4 \times 2 \times 10^4 = \boxed{(2)} \text{ (cells/mL)}$$

【滑膜幹細胞懸濁液の総細胞濃度】

$$\boxed{(1)} + \boxed{(2)} = \boxed{(3)} \text{ (cells/mL)}$$

【滑膜幹細胞の生存率】

$$\boxed{(1)} \div \boxed{(3)} \times 100 = \boxed{(4)} \text{ (\%)}$$

【回収した総細胞数】

$$\boxed{(3)} \text{ cells/mL} \times 20 \text{ mL} = \boxed{(5)} \text{ (cells)}$$

