O'Pet 萬寶特生醫

Daily care for your pets

動物退化性關節炎分期、診斷及脂肪間質幹細胞在退化性關節炎與軟組織修復之應用

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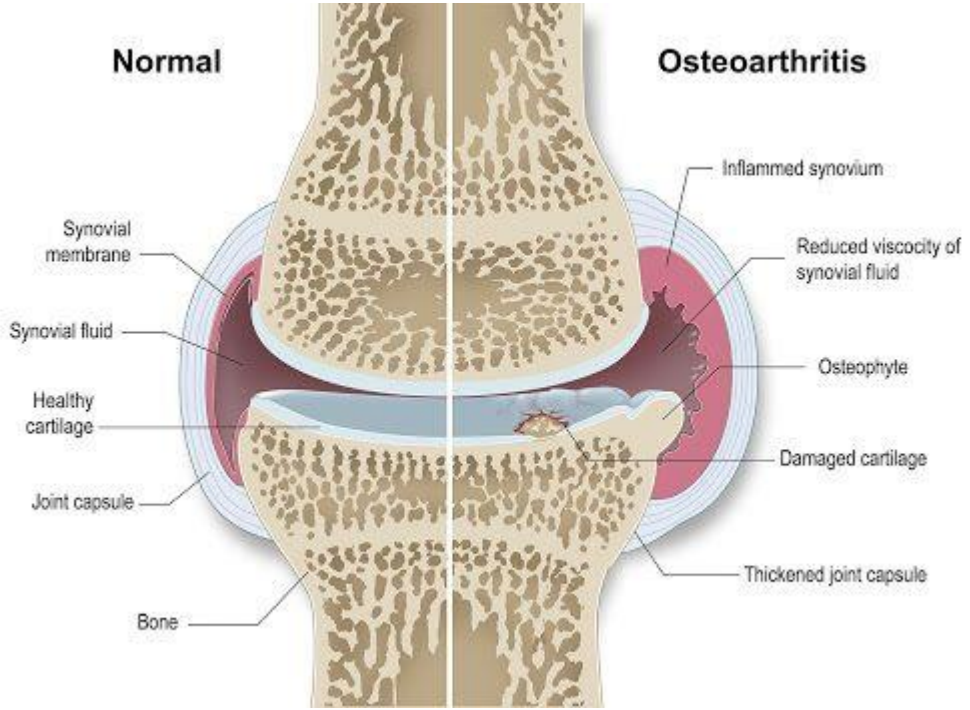
十六項專利發明人 (十項脂肪間質幹細胞相關國際專利)
第十四屆國家新創獎得主

演講內容

1. 簡介退化性關節炎及其成因
2. 造成退化性關節炎的危險因子及呈現的訊號
3. 退化性關節炎的分類分級(COAST-2018)
4. 當下退化性關節炎的治療模式
5. 脂肪間質幹細胞應用於退化性關節炎的治療

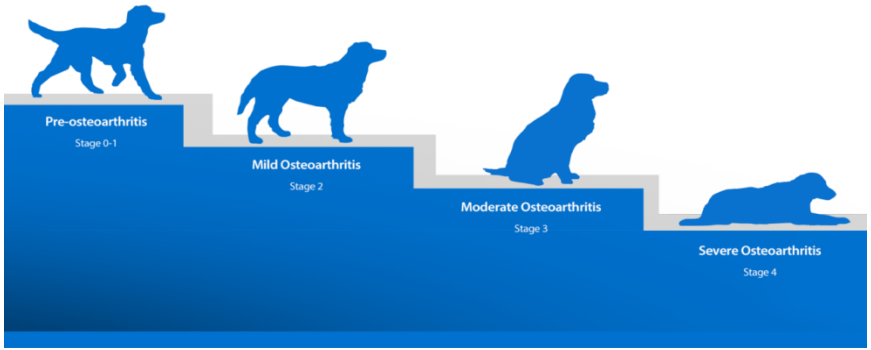
關節結構與退化性關節炎



退化性關節炎

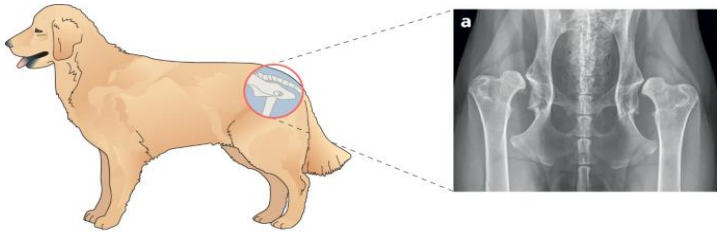
膝關節在長期受力下，關節軟骨退化磨損，可能形成骨刺、關節變形、失去彈性，而產生關節疼痛、僵硬、以致於影響活動功能。

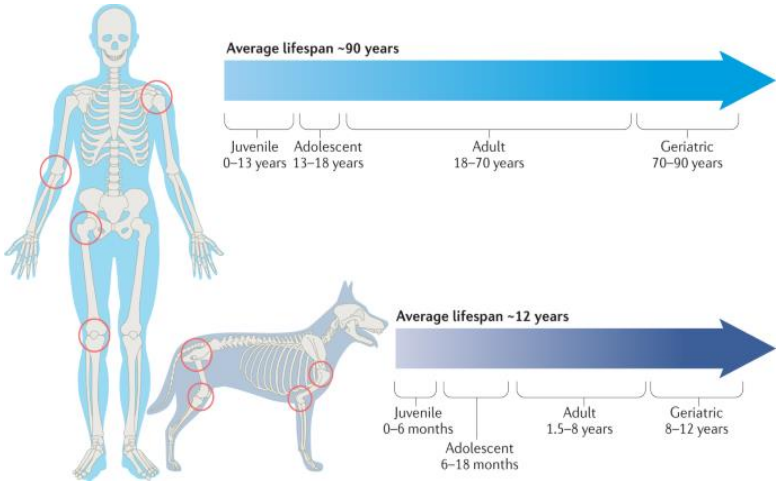
（一）外傷：膝關節損傷、骨折、韌帶損傷都會加速退化。
（二）先天性骨骼異常或關節受力不平衡：姿勢不良引起肌肉柔軟度變差，或是肌肉力量下降而造成關節磨損。
（三）肥胖：體重造成關節過度負荷

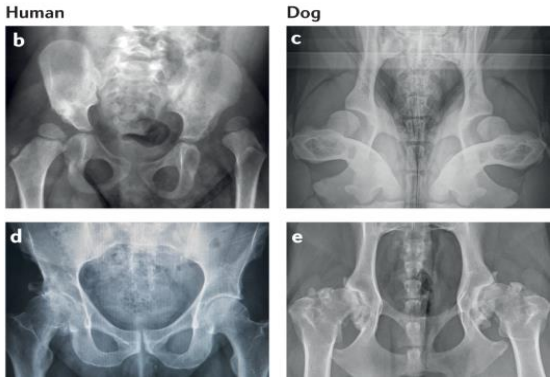


	 大型犬	 中小型犬	 老年犬	 肥胖犬
髖關節發育不全	✓			✓
膝蓋骨異位	✓ 外側	✓ 內側		✓
肘關節水囊腫	✓		✓	✓
肘關節脫臼		✓		✓
退化性關節炎	✓	✓	✓	✓

有關節處
就有機會發生關節炎







犬隻發生退化性關節炎的危險因子

每隻狗都會得到退化性關節炎特別是年紀的因素。但某些因素將讓寵物更容易罹患這樣的病症，這些因素包括：

1. 大量繁殖的犬種如:德國狼犬 German Shepherd Dogs,拉不拉多 Labrador Retrievers 及黃金獵犬 Golden Retrievers
2. 肥胖
3. 年紀：中年及老年犬
4. 運動造成的重複性壓力如 敏捷性訓練，接飛球，高處跳下...
5. 骨折或韌帶斷裂
6. 感染誘發關節疾病如: 萊姆氏症Lyme Disease
7. 錯誤的飲食
8. 不良的居住環境
9. 基因

犬隻出現退化性關節炎的訊號 (Signs of Osteoarthritis in Dogs)

四肢僵直，跛行或起身困難 (Stiffness, lameness, or difficulty getting up)
嗜睡 (Lethargy)
勉強的跑 跳或玩 (Reluctance to run, jump, or play)
體重上升 (Weight gain)
易怒或改變生活習慣 (Irritability or changes in behavior)
拍拍或觸摸時伴隨疼痛 (Pain when petted or touched)
難以維持上廁所的姿勢或常在室內發生意外 (Difficulty posturing to urinate or defecate, or having accidents in the house)
四肢或脊椎處肌肉量消失 (Loss of muscle mass over the limbs and spine)

退化性關節炎評估量表

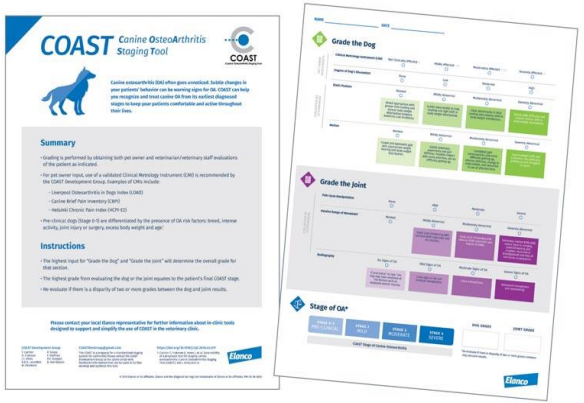
WSAVA Global Veterinary Community

GUIDELINES FOR RECOGNITION, ASSESSMENT AND TREATMENT OF PAIN

Canine Brief Pain Inventory.
http://www.vet.upenn.edu/research/clinicaltrials/vcic/pennchart/cbpi-tool

Helsinki Chronic Pain Index by University of Helsinki.
http://www.vetmed.helsinki.fi/english/animalpain/hcpi/

JSSAP Canine Chronic Pain Index.
http://www.dourinken.com/itami.htm



Canine Brief Pain Inventory (CBPI)

Description of pain:
Rate your dog's pain:

1. Fill in the oval next to the one number that best describes the pain at its **worst** in the last 7days.

O0 O1 O2 O3 O4 O5 O6 O 7 O 8 O 9 O 10
No pain — Extreme pain

2. Fill in the oval next to the one number that best describes the pain at its **least** in the last 7 days

O0 O1 O2 O3 O4 O5 O6 O 7 O 8 O 9 O 10
No pain — Extreme pain

3. Fill in the oval next to the one number that best describes the pain at its **average** in the last 7 days.

O0 O1 O2 O3 O4 O5 O6 O 7 O 8 O 9 O 10
No pain — Extreme pain

4. Fill in the oval next to the one number that best describes the pain as it is **right now**.

O0 O1 O2 O3 O4 O5 O6 O 7 O 8 O 9 O 10
No pain — Extreme pain

Description of function:
Fill in the oval next to the one number that best describes how during the last 7 days **pain has interfered** with your dog's:

5. **General Activity**

O0 O1 O2 O3 O4 O5 O6 O 7 O 8 O 9 O 10
Does not interfere — Completely interferes

6. **Enjoyment of Life**

O0 O1 O2 O3 O4 O5 O6 O 7 O 8 O 9 O 10
Does not interfere — Completely interferes

7. **Ability to Rise to Standing From Lying Down**

O0 O1 O2 O3 O4 O5 O6 O 7 O 8 O 9 O 10
Does not interfere — Completely interferes

8. **Ability to Walk**

O0 O1 O2 O3 O4 O5 O6 O 7 O 8 O 9 O 10
Does not interfere — Completely interferes

9. **Ability to Run**

O0 O1 O2 O3 O4 O5 O6 O 7 O 8 O 9 O 10
Does not interfere — Completely interferes

10. **Ability to Climb Stairs, Curbs, Doorsteps, etc.**

O0 O1 O2 O3 O4 O5 O6 O 7 O 8 O 9 O 10
Does not interfere — Completely interferes

Overall impression:

11. Fill in the oval next to the one number that best describes your dog's overall quality of life over the last 7 days.

O Poor O Fair O Good O Very Good O Excellent

Canine Brief Pain Inventory	
Severity Domain	**Interference Domain**
Worst Pain	General Activity
Least Pain	Enjoyment of Life
Average Pain	Rising to Standing
Pain Now	Walking
	Running
	Climbing

Helsinki Chronic Pain Index (HCPI)

Dog's Name: Date:

Helsinki Chronic Pain Index

Hielm-Bjorkman HK, Rita H, Tulamo R-M. Psychometric testing of the Helsinki chronic pain index by completion of a questionnaire in Finnish by owners of dogs with chronic signs of pain caused by osteoarthritis. Am J Vet Res. 70: 727 – 734, 2009.

(As translated from Finnish to English)

Circle the pain and function description that best represents your dog's behaviour:

Rate your dog's attitude and/or mood:

0	1	2	3	4
Very alert	Alert	Neither alert nor disinterested	Disinterested	Very disinterested/ lethargic

Rate your dog's willingness to participate in play or interact:

0	1	2	3	4
Very willing	Willing	Reluctant	Very reluctant	Does not participate or interact at all

Rate your dog's frequency in vocalization or discomfort behaviour (audible whining, grunting, yelping, or unusual licking):

0	1	2	3	4
Never	Hardly ever	Sometimes	Often	Very often

Rate your dog's eagerness to walk:

0	1	2	3	4
Very eager	Eager	Reluctant	Very reluctant	Does not want to walk at all

Rate your dog's ability and/or willingness to walk up and/or down stairs:

0	1	2	3	4
Very willing/able	Willing/able	Reluctant	Very reluctant	Does not do stairs at all

Dog's Name: Date:

Helsinki Chronic Pain Index con't

Rate your dog's ability and/or willingness to run:

0	1	2	3	4
Very willing/able	Willing/able	Reluctant	Very reluctant	Does not run at all

Rate your dog's ability and/or willingness to jump (onto bed, couch, vehicle, etc):

0	1	2	3	4
Very willing/able	Willing/able	Reluctant	Very reluctant	Does not jump at all

Rate your dog's ease in lying down:

0	1	2	3	4
Very easy	Easy	Neither easy nor difficult	Difficult	Very difficult

Rate your dog's rising from a down position:

0	1	2	3	4
Very easy	Easy	Neither easy nor difficult	Difficult	Very difficult

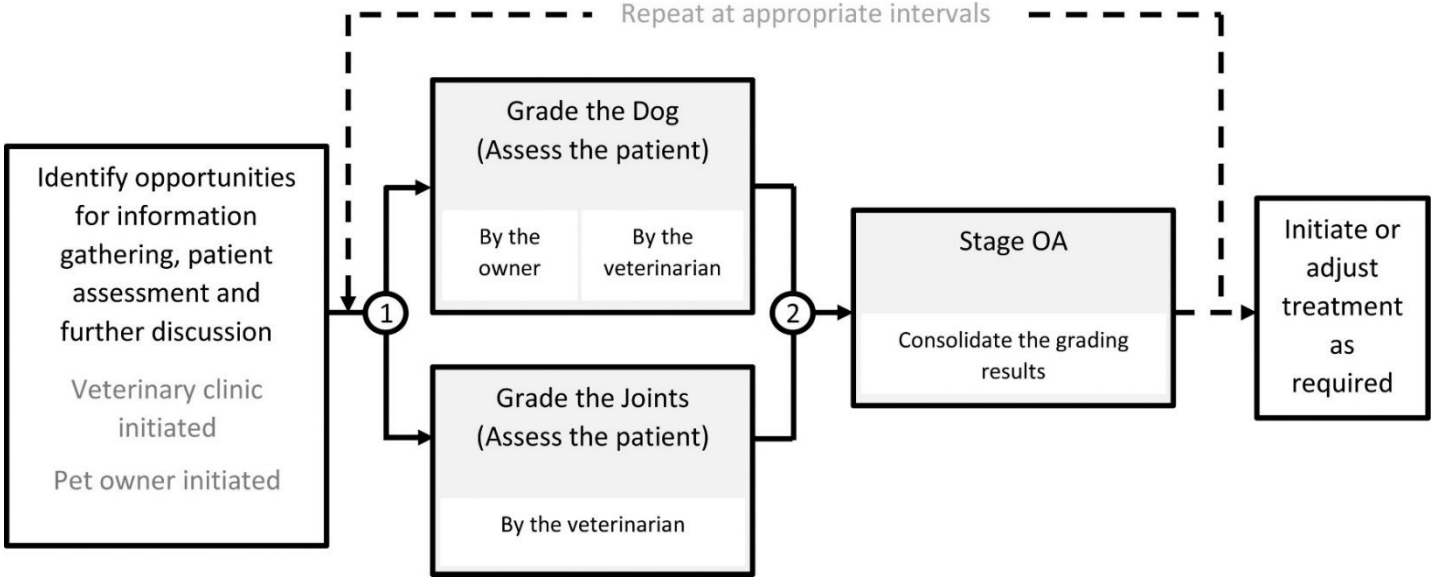
Rate your dog's ease of movement after a long rest:

0	1	2	3	4
Very easy	Easy	Neither easy nor difficult	Difficult	Very difficult

Rate your dog's ease of movement during and/or after exercise/walks (tired, dragging feet, scuffing nails, lying down):

0	1	2	3	4
Very easy	Easy	Neither easy nor difficult	Difficult	Very difficult

退化性關節炎量表--飼主與醫師雙向評估法 (COAST)



Face validity of a proposed tool for staging canine osteoarthritis: Canine OsteoArthritis Staging Tool (COAST)

T. Cachon et al. The Veterinary Journal 235 (**2018**) 1–8

COAST的好處

Potential advantages of a standardised staging system for canine osteoarthritis (OA; e.g. Canine OsteoArthritis Staging Tool).

General advantages of an OA staging system	Potential additional benefits
Standardised approach to assessment 標準評估流程	Improved transfer of information between veterinarians Harmonised approach/consistency in advice provided to pet owners Consistency in evaluation and re-evaluation of canine patients
Record keeping/log of disease severity 疾病程度的紀錄	Guide for healthcare plan and decision making Improved evaluation of response to treatment Precise monitoring of disease progression
Enhanced monitoring of dogs at risk of developing OA 增強退化性關節炎發展之危險因子管理	Increased pet owner awareness of canine OA Improved pet owner understanding of the disease (including benefits of regular assessment and early detection) Engaged pet owners as part of the disease management team
Encompasses early detection of OA 包含退化性關節炎的早期偵測	Provision of best standard of care from the earliest clinical signs of OA Optimised well-being of the dog at that time Potential for improved disease management (minimisation of pain, disability and behavioural problems later in life)
Optimised care 照護的最大化	Maximised health and welfare benefits for the dog Strong partnership between the veterinary clinic and pet-owner Job satisfaction

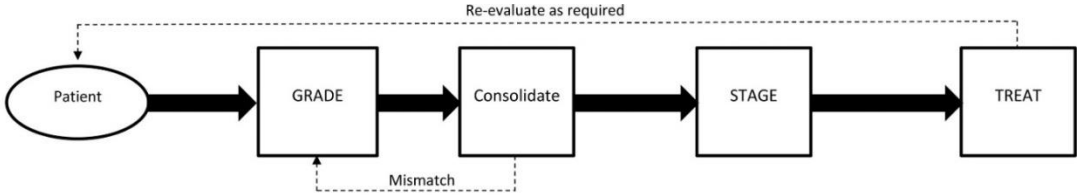
與飼主組成健康照護團隊

運用院內護士、技術員或接待員向飼主(特別是新手飼主)
簡介及說明造成退化性關節炎的危險因子

Opportunities within first opinion practice to assess dogs at risk of developing osteoarthritis.

Risk factor	Assessment opportunity
Genetic predisposition (developmental orthopedic disease)	Preventative clinic
Intense activity	General health assessment of athletes/dogs with very active lifestyle
Traumatic joint injury or joint surgery	Post injury/surgery assessment
Excess body weight	Obesity/weight management clinics
Age	Geriatric clinics
All of the above	Annual general wellness assessments

An overview of the whole COAST process



Opportunities for patient assessment

GRADE THE DOG			
CMI	Discomfort	Posture	Motion

GRADE THE JOINT		
Pain upon manipulation	Physical examination	Radiographs

STAGING			
Most severe grade	Description		COAST
	Preclinical	Clinically normal. No OA risk factors	0
		Clinically normal. but "OA risk"	1
	Mild clinical signs		2
	Moderate clinical signs		3
	Severe clinical signs		4

Initiate or adjust treatment as required

Mismatch

使用COSAT進行分類分級 STEP-1

CMI:
LOAD
CBPI
HCPI
JSSAP-CCPI

Canine OsteoArthritis Staging Tool (COAST) assessment parameters for 'Grade the dog'.

Pet owner observations (Home/non-clinic environment)				Evaluations by a veterinarian (Veterinary clinic)		
Clinical metrology instrument	**Degree of dog's**	**Effect on**	**Additional descriptors**	**Effect on**	**Additional descriptors**	
0 or very low score clinically affected ☐						
Low score or 'mild affected' ☐						
Medium score or 'moderately affe ☐						
High score or 'severely affected' ☐	Unbearable ☐	Severely abnormal ☐	Restless when standing Reluctance (difficulty) to stay standing Severe shift in static body weight distribution Severely abnormal limb loading	Severely abnormal (very obvious changes) ☐	Struggles to move/reluctant to move Severe lameness usually present Severe weight shift Marked difficulty rising (getting up)	

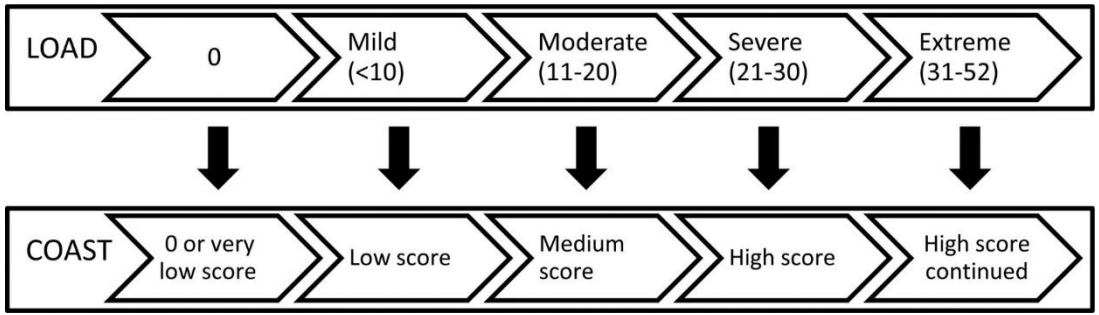
Pet Owner		Veterinarian	
CMI	**Discomfort**	**Posture**	**Motion**
☐	☐	☐	☐
☐	✓	☐	☐
✓	☐	✓	✓
☐	☐	☐	☐

Less severe

Most severe

Duration for 1 month

Extrapolating Liverpool Osteo-Arthritis in Dogs (LOAD) scores to the Canine Osteo-Arthritis Staging Tool (COAST) clinical metrology instruments (CMI) scoring system.

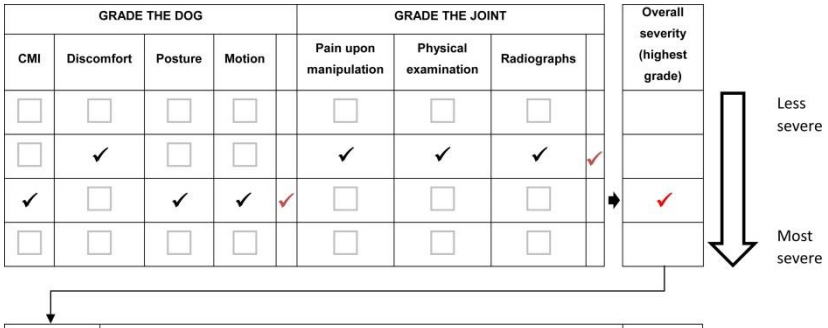


使用COSAT進行分類分級 STEP-2

Canine OsteoArthritis Staging Tool (COAST) assessment parameters for 'Grade the joint. Ideally each joint should be graded so that disease progression can be independently and accurately monitored over time. The evaluation chart can be annotated to identify the joint being assessed.

Evaluations by a veterinarian Veterinary clinic				
Pain upon manipulation	**Passive range of movement**	Additional descriptors	**Radiography**	Additional descriptors
None ☐	Normal ☐	Normal	No radiographic signs of OA ☐	If preclinical 'at risk', the dog may have radiographic evidence of risk factors such as dysplasia and/or trauma
Mild ☐	Mildly abnormal ☐	Minimally reduced ROM No crepitus Slight joint thickening	Mildly abnormal (subtle changes) ☐	Early signs of OA Minimal osteophytes
Moderate ☐	Moderately abnormal ☐	Obvious decrease in ROM Muscle atrophy Obvious joint thickening	Moderately abnormal (obvious changes) ☐	Obvious osteophytes
Severe ☐	Severely abnormal ☐	Extremely limited ROM Crepitus Extreme muscle atrophy Severe joint thickening Loss of anatomical normality upon palpation Anatomical misalignment	Severely abnormal (very obvious changes) ☐	Advanced osteophytes Remodeling

☐ = check mark box.



Overall severity	Description		STAGE of OA
	PRECLINICAL	No risk factors apparent	0
		"At risk": At least one predisposing factor for OA apparent e.g. breed predisposition, joint injury, obesity, intense activity and/or radiographic signs of dysplasia or joint trauma	1
	MILD		2
✓	MODERATE		3
	SEVERE		4

Staging of canine osteoarthritis (OA) with the Canine OsteoArthritis Staging Tool (COAST). The stage of OA equates to the highest grade for any of the parameters assessed. Classification of preclinical dogs as either stage 0 or 1 requires consideration of risk factors.

Description		Stage of OA
Preclinical	No risk factors apparent	0
	'At risk': At least one predisposing factor for OA apparent e.g. breed predisposition, joint injury, obesity, intense activity and/or radiographic signs of dysplasia or joint trauma	1
Mild		2
Moderate		3
Severe		4

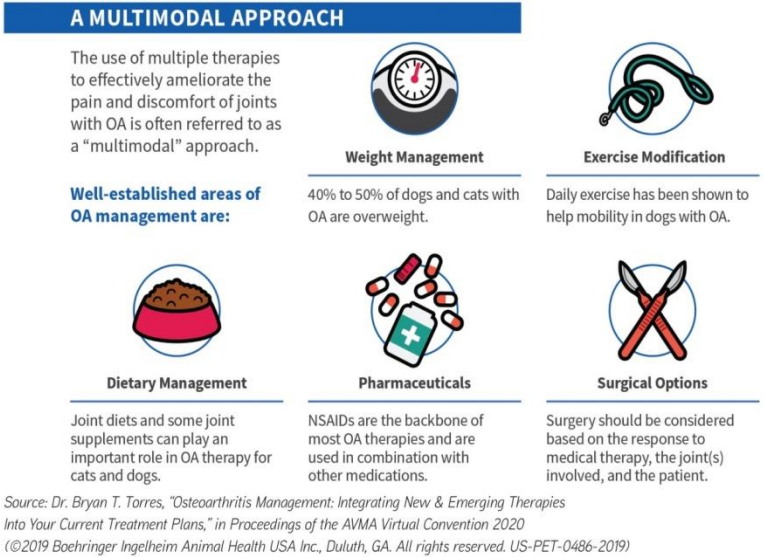
Overall Grade for the Dog	Overall Grade for the Joints	Overall Disease Severity	Stage of OA	
☐	☐	☐	0	
☐	☐	☐	1	
☐	✓	☐	2	⇦
☐	☐	?	3	"Mismatch"
✓	☐	?	4	⇦

Example of a 'mismatch'. Difference of two grades between the grade of the dog and the grade of the joints. Overall disease severity and stage of osteoarthritis. cannot be immediately established.
Recommendation: re-evaluate the dog and consider other diseases.

退化性關節炎的治療 Treatment of Osteoarthritis

獸醫師面對犬隻有髖關節或肘關節的異常，
常用的建議治療方式可能包括：

1. 止痛 (Pain management)
2. 減重與營養管理 (Weight loss and a nutritional plan)
3. 常態性低衝擊性運動 (Regular, low-impact exercise)
4. 提供Omega 3 脂肪酸 (Omega 3 fatty acids)
5. 保護犬隻關節的輔具 (Supplements to protect your dog's cartilage)
6. 復健 (Physical rehabilitation and therapeutic modalities)
7. 開刀 (Surgery)
8. **關節腔注射 (Intra-articular injections)**
9. 針灸及其他輔助治療 (Acupuncture and other complementary therapies)



Source: Dr. Bryan T. Torres, "Osteoarthritis Management: Integrating New & Emerging Therapies Into Your Current Treatment Plans," in Proceedings of the AVMA Virtual Convention 2020

退化性關節炎的治療 Treatment of Osteoarthritis

1. **類固醇**：過去類固醇曾被大量使用於關節炎的患者，類固醇的止痛效果快速顯著，但是消化道出血、皮膚變薄、骨質疏鬆症、傷口不易癒合等副作用也相當大，甚至會加重高血壓、糖尿病等疾病程度。
2. **非類固醇抗炎藥**：這一群的藥物有很多，目前相當普遍，止痛效果也都不錯，但長期使用易產生消化性潰瘍、下肢水腫、腎臟功能損傷等副作用。目前市面上有新的非類固醇抗發炎藥物(COX2)，對腸胃的刺激性大大地降低，也減少了長期服用所帶來的副作用。新一代的藥物，比較不會胃痛，不過要有胃潰瘍的病史或六十歲以上才可以開立。現在有些消炎貼布可在膝蓋局部使用也有相同的效果且不會傷胃。
3. **葡萄糖胺（Glucosamine）**：葡萄糖胺可刺激關節內軟骨細胞合成醣蛋白，而且也有消炎止痛效果，卻沒有非類固醇抗炎藥的副作用。在台灣行政院衛生署核准適應症為「因骨關節代謝機能衰退引發之關節病如頸關節炎、髂關節炎、肩胛關節炎、膝關節炎、背關節炎、骨質疏鬆、骨膜硬化、腰痛、骨折、骨關節營養不良、慢性和亞急性關節炎」。健保署已取消給付

退化性關節炎的治療 Treatment of Osteoarthritis

4. **玻尿酸製劑** 注射玻尿酸，可以使患部減少的關節液獲得補充，既可包覆、潤滑軟骨表層，又可滲入基層抑制軟骨退化，同時改善關節活動度，也有止痛、消炎的作用。雖然玻尿酸雖具有其之功效，但各相關專業人士對於此藥有不同意見
5. **復健治療** 適當的肌力訓練可以減輕關節的負擔，減少引發疼痛的活動，對於骨關節炎也是有很大的幫助。
6. **手術治療** 當使用一些保守性療法如：休息、藥物、復建等都沒辦法消除疼痛時，就需要開刀治療。開刀的方法很多，外科手術治療包括關節鏡手術、截骨矯正手術、人工關節置換等。視症狀的嚴重程度，予以不同的治療。若是單純的只是軟骨的輕微磨損、關節腔游離物及滑液膜發炎，這時可用關節鏡對關節進行清創的工作。截骨矯正手術是將變形的骨頭作矯正，讓已經被破壞的軟骨部分能休息。若是膝關節嚴重退化，幾乎所有關節軟骨都破壞殆盡時，就要考慮施行人工膝關節置換術。
7. **脂肪間質幹細胞進行關節腔注射**，具有消炎、止痛作用，並有部分軟骨修復功能，具有長時間改善動物生活品質的效果。

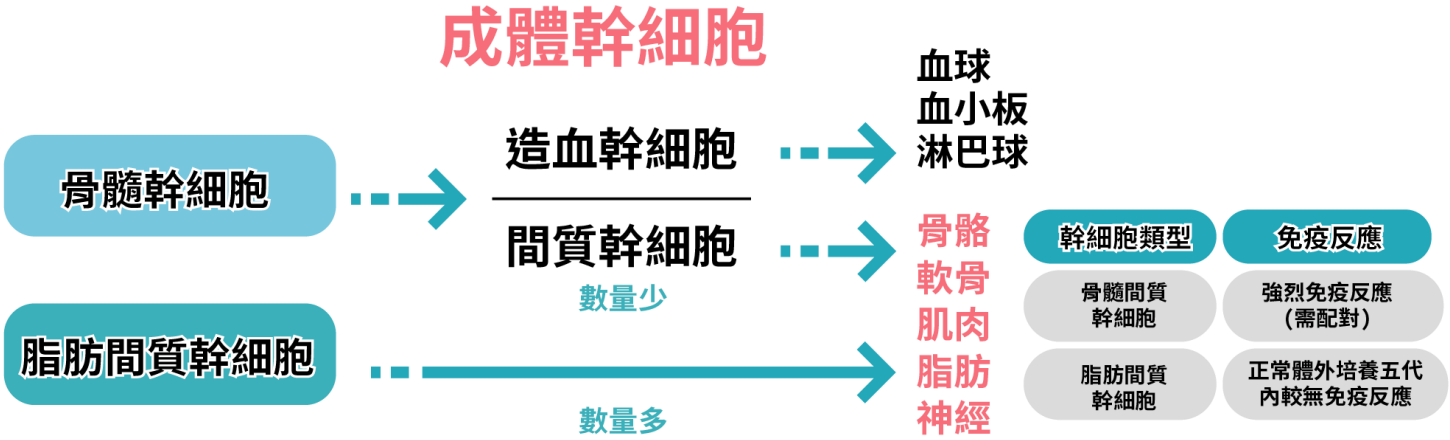
脂肪間質幹細胞應用於退化性關節炎的治療

一個治療的成功取決於多項因素：

1. 飼主的配合度
2. 治療用品的品質
3. 醫師的診斷與技術

什麼是幹細胞? 為何用脂肪間質幹細胞?

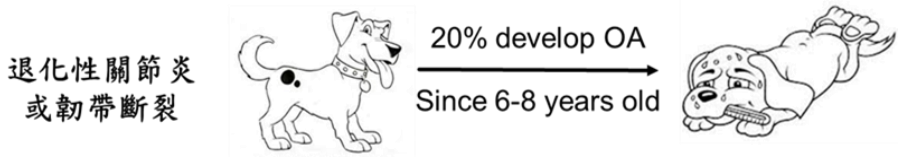
一種未分化的細胞，經適當的誘發(激素或環境)可形成體內的多種組織。越原始的幹細胞(如胚胎幹細胞)在生物體的應用，就越有產生畸胎瘤(Teratoma)的風險。



脂肪間質幹細胞在獸醫學的應用

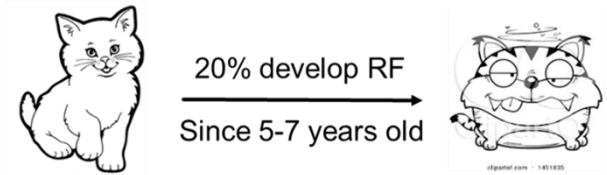
Cartilage lesions and cartilage degeneration and tendon lesions 軟骨韌帶修復
Reed S, Leahy E. J Anim Sci 2013; 1: 59-65.
Nixon A, et al. Am J Vet Res 2008; 7: 928-937.

Osteoarthritis 退化性關節炎
Black L, et al. Vet Ther 2007; 4: 272-284.
Guercio A, et al. Cell Biol Int 2012; 2: 189-194.



IVDD 椎間盤疾病
Sakai D, et al. Biomaterials. 2003;24(20):3531–3541.
Yang F, et al. Molecular Therapy. 2009;17(11):1959–1966.

Chronic Kidney Disease 慢性腎衰竭
Boyd LM, et al. J Vet Intern Med 2008;22:1111–1117
Quimby JM, et al. Vet J. 2015 Jun;204(3):241-6.



Central or peripheral nervous system lesions 中樞與周邊神經修復
Ryu H, et al. J Vet Sci 2009; 4: 273-284.
Ghoreishian M, et al. J Oral Maxillofac Surg 2013; 3: 577-587.

Treatment of cardiomyopathies 心臟疾病治療
Pogue B, et al. J Small Anim Pract 2013; 7: 361-366.

未來目標：脊椎損傷（四、五級），肝硬化，中風，心肌壞死，眼角膜損傷…等

Outcome of Allogeneic Adult Stem Cell Therapy in Dogs Suffering from Osteoarthritis and Other Joint Defects

Shah et al. Stem Cells International, Volume 2018, Article ID 7309201, 7 pages

Grading	Radiographic features	OA
Grade 0	No abnormalities	No features of OA
Grade 1	Minute osteophytes	Doubtful
Grade 2	Definite osteophytes	Minimal
Grade 3	Diminished joint space	Moderate
Grade 4	Greatly diminished joint space + sclerosis of the subchondral bone	Severe

*Adapted from Arden and Nevitt [25], *Best Practice and Research Clinical Rheumatology*.

Age group	Grade 2	Grade 3	Grade 4
A: 0–5 years	14	14	14
B: 6–9 years	15	40	6
C: 10–13 years	3	42	16
D: 14–16 years	0	7	22

Lameness	Pain	Functional disability
1 (normal)	1 (no pain)	1 (normal)
2 (intermittent)	2 (mild pain)	2 (slightly stiff)
3 (persistent)	3 (severe pain)	3 (stiff)
4 (non-weight bearing)	4 (severe pain)	4 (very stiff, unwilling to walk)
5 (ambulatory only with assistance)	5 (severe pain)	5 (need assistance to walk)

#Adapted from Black et al. [6].

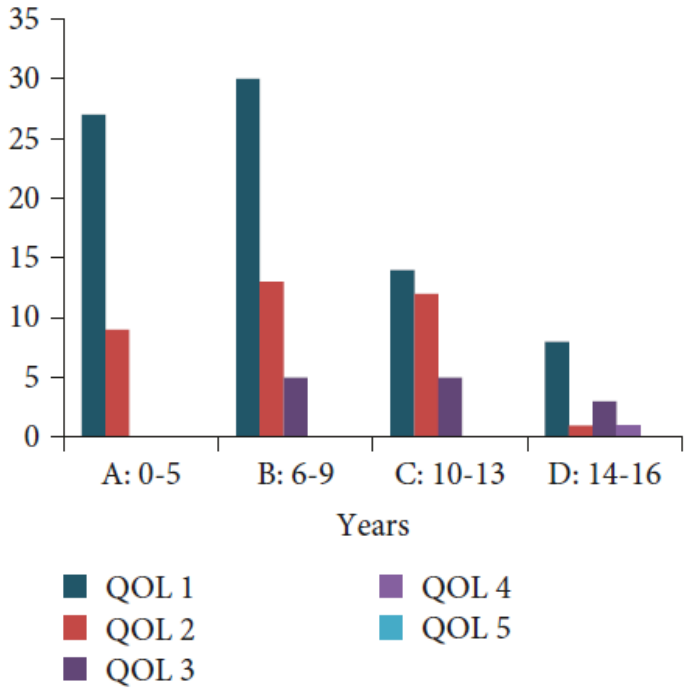
Score	Symptoms
1	Excellent improvement
2	Good improvement
3	No difference
4	Worse
5	Considerably worse or death

Table 5: IA injection and quality of life score with respect to the age groups.

Age group	QOL 1	QOL 2	QOL 3	QOL 4	QOL 5
A: 0–5 years	27	9	0	0	0
B: 6–9 years	30	13	5	0	0
C: 10–13 years	14	12	5	0	0
D: 14–16 years	8	1	3	1	0

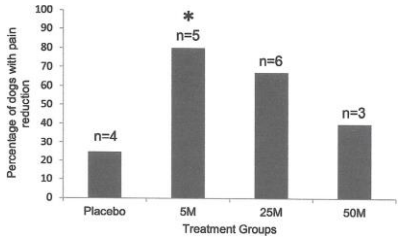
Table 6: IV injection and quality of life score with respect to the age groups.

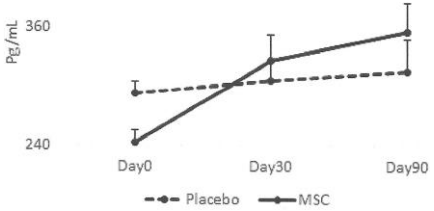
Age group	QOL 1	QOL 2	QOL 3	QOL 4	QOL 5
A: 0–5 years	3	1	2	0	0
B: 6–9 years	5	4	4	0	0
C: 10–13 years	14	11	5	0	0
D: 14–16 years	3	9	4	0	0

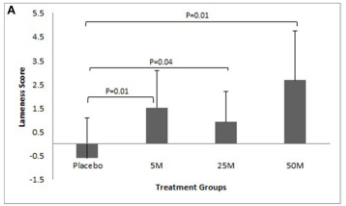


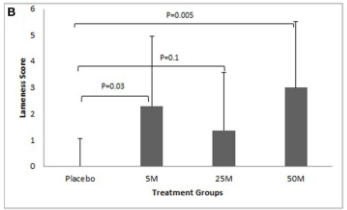
Intra-articular Administration of Allogeneic Adipose Derived MSCs Reduces Pain and Lameness in Dogs With Hip Osteoarthritis: A Double Blinded, Randomized, Placebo Controlled Pilot Study

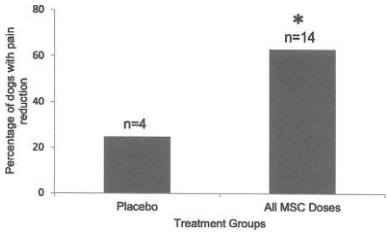
Chad B. Maki et al. **Frontiers in Veterinary Science**, August 2020 | Volume 7 | Article 570

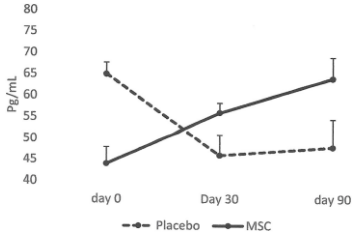


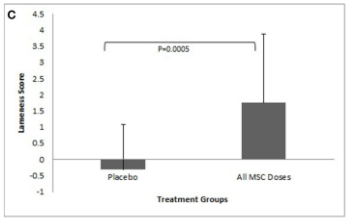






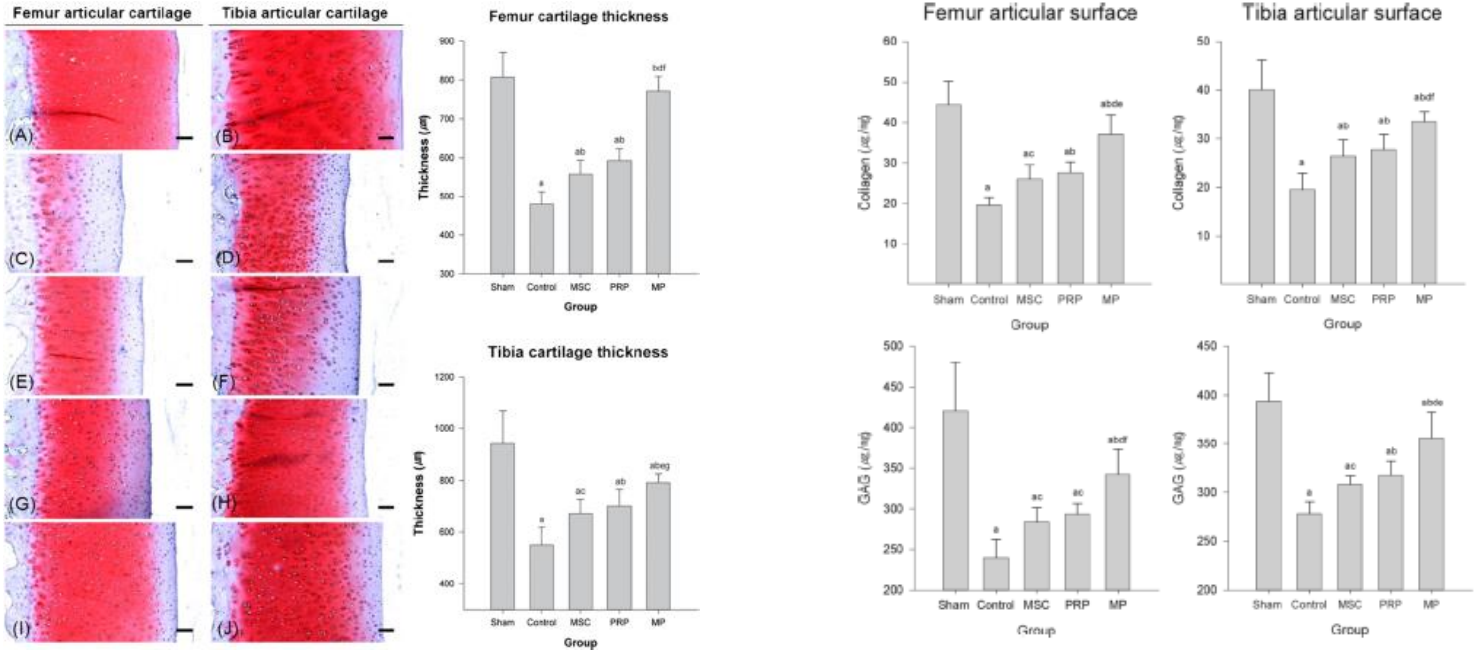






Adipose-derived mesenchymal stem cells and platelet-rich plasma synergistically ameliorate the surgical-induced osteoarthritis in Beagle dogs

Yun et al. Journal of Orthopaedic Surgery and Research (2016) 11:9



A Regenerative Approach to Canine Osteoarthritis Using Allogeneic, Adipose-Derived Mesenchymal Stem Cells. Safety Results of a Long-Term Follow-Up

Éva Kriston-Pál et al. **Frontiers in Veterinary Science**, August 2020 | Volume 7 | Article 510

TABLE 1 | Summary of long-term follow-up after MSC transplantation.

Site of OA	No. of animals included/No. of animals evaluated	Range of age (average) at the time of MSC injection in years	Average age at the time of follow-up (years)	Results at 2.5, 4, or 5-year follow-up		Joint or spinal diseases other than the transplanted joint	Number and cause of death	
				Improved or sporadic lameness and/or medication during weather fronts or extreme activity	No improvement continuous medication for other joint/spinal problems or death		Other than cancer	Cancer
Elbow OA	39*/31**	0.6–10 (3)	7.3	26 (84%)	5	9	8	3
Hip OA	5/4[&][&]	1.5–8 (5.5)	9.7	3	1	2	1	1
Knee OA, dislocation, ligament tear	8/8[&][&]	3–10 (5.6)	10	5 1	1	3	3	-
Hock OA	1	0.8	5	1	-	-	-	-
Ankle OA	2	0.4–1.5 (0.96)	5.5	2	-	-	-	-
Total	55[&]/44[&][&]	0.4–10 (3.2)	7.5	37 (84%)#	7 (16%)	14	12	4

The condition (lameness, usage of drugs) of the transplanted joint of the same animal was followed up for 2.5, 4, or 5 years depending on the date of transplantation.

 # % = (improved animal number: number of animals in the study) × 100.

**6 dogs died within 1 year of the survey.*

***3 of them died just before the end of the survey, but their condition was evaluated till death.*

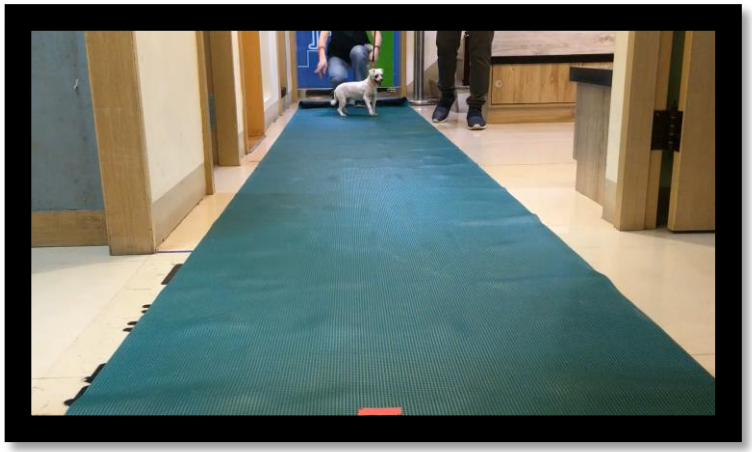
[&]One dog died within 1 year of the survey and 3 died later but before the end of the follow-up.

[&][&]One dog who died before the end of the survey was evaluated till death.

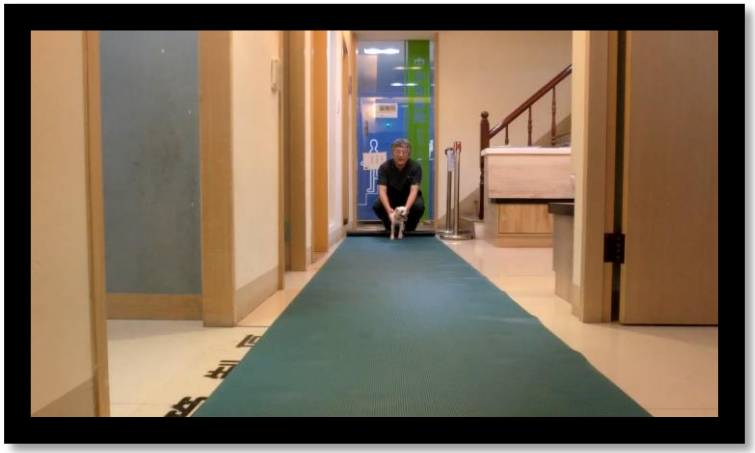
Conclusions

1. Our data presented in this paper suggest that **MSC transplantation results in improvement of motion**. The results showed that **83%** of the OA patients improved or retained improvement in lameness.
2. Osteoarthritis cases in joints other than the elbow (knee, hip, ankle, and hock) were also evaluated. Eighty four percentage of the evaluated dogs **retained their improved condition after the 4-5-year follow-up**.
3. MSC transplantation **does not appear to be associated with an increase in malignancies or other diseases**, and **no other adverse effects emerged due to MSC injection**. These findings are underpinned by: (1) the published literature that supports that **MSC is not a tumorigenic cell type**; (2) published findings that local injection into the joint results in the **adherence of MSCs to the damaged cartilage** with no reported evidence for their migration outside of the treated joint.

寵物退化性關節炎治療



幹細胞治療注射前



幹細胞治療注射後1個月

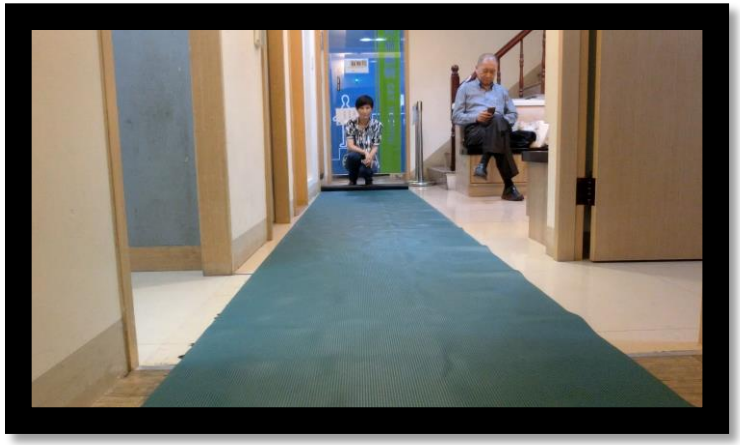
寵物退化性關節炎治療

幹細胞治療注射前

幹細胞治療注射後1個月

寵物十字韌帶斷裂治療

幹細胞治療注射前



幹細胞治療注射後2個月

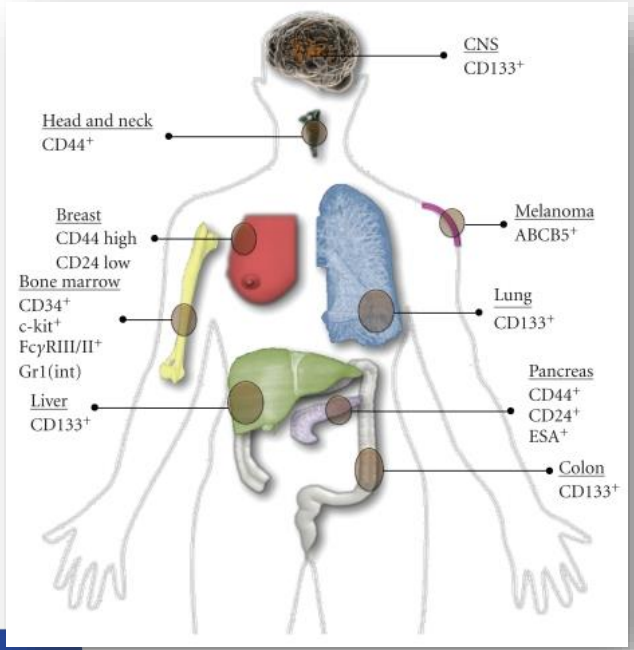
國際間質幹細胞協會對於間質幹細胞的定義

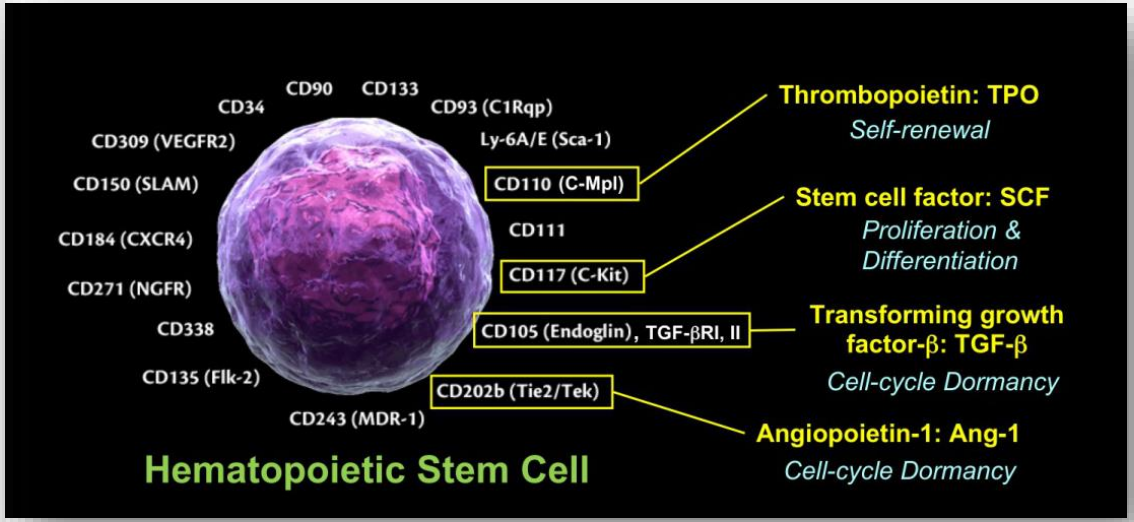
1. 間質幹細胞在標準培養程序下須能貼附於培養盤上
2. 間質幹細胞必須表現以下細胞表面標記CD105, CD73 and CD90,
   但不得表現 CD45, CD34, CD14 or CD11b, CD79a or CD19 and HLA-DR.
3. 間質幹細胞在體外必須能被誘導分化成為造骨細胞，脂肪細胞與軟骨細胞

by the Mesenchymal Tissue Stem Cell Committee of the International Society for Cellular Therapy (Dominici et al: Cytotherapy, 2006)

細胞表面抗原為何如此重要?

Stem cell markers are genes and their protein products used by scientists to isolate and identify stem cells. Stem cells can also be identified by functional assays.





Recent advances in hematopoietic stem cell gene therapy.
By Tochihisa Tsuruta. 2013, Innovations in stem cell transplantation

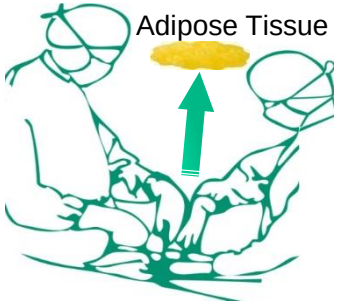
何種因素會改變細胞表面抗原?

1. 化學因素: Growth factors, Toxin…
2. 物理因素: Culture environment, CO_2 concentration, Culture plate …
3. 細胞生理: Age, Passage…

這些變數造就了細胞治療的不穩定性…
如何維持並提供穩定的間質幹細胞?

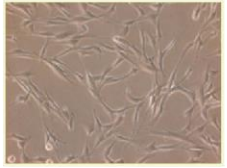
骨萬寶®
脂肪間質幹細胞處理流程

GTP-like Lab



從組織中分離細胞

篩選純化

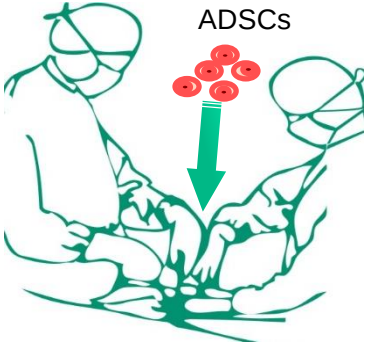


體外培養放大

凍存

解凍

提供獸醫院



檢測分離之脂肪幹細胞的分化能力

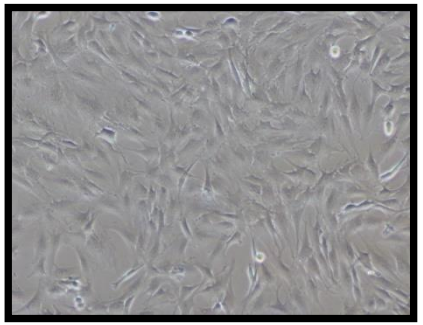
ADSCs

↓

Differentiation
Ability test

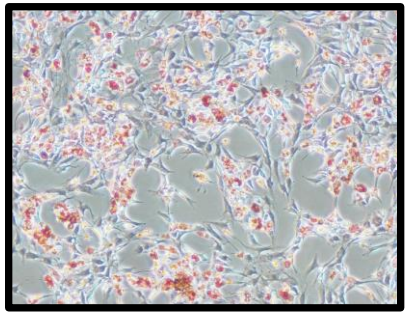
分離後的間質細胞，
經幹細胞篩選培養及培養後，
經不同誘發分化培養基培養
兩周後證實具有骨分化、
軟骨分化及脂肪分化能力

100x



2 weeks

Adipo-
medium



100x

Adipogenesis (Oil Red)

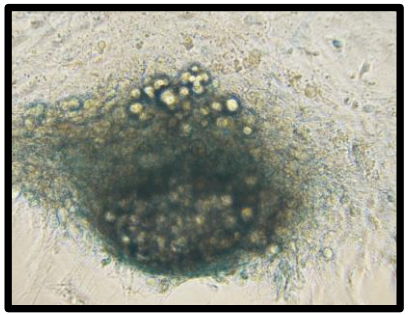
Chondro
-medium

2
weeks

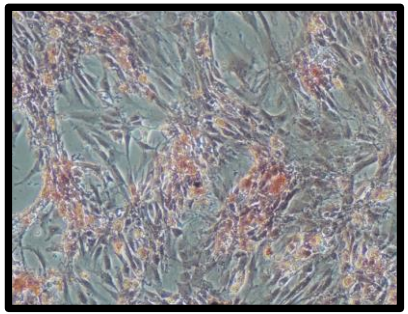
2 weeks

Osteo-
medium

200x



Chondrogenesis (Alcian Blue)



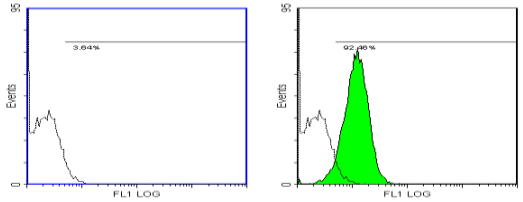
100x

Osteogenesis (Alizarin Red)

檢測分離之脂肪幹細胞的表面抗原

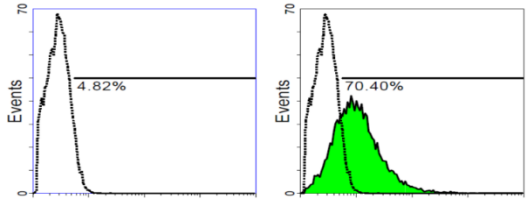
High expression

CD271⁺



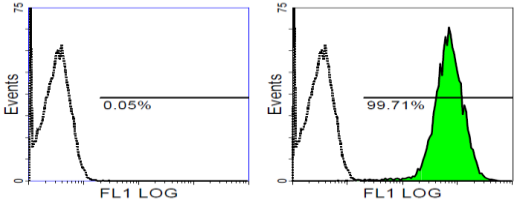
IgG2a isotype control　　CD271-FITC

CD73⁺



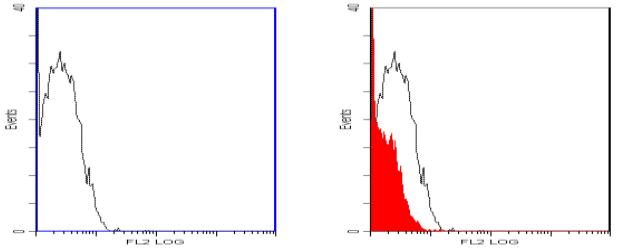
IgG2a isotype control　　CD73-FITC

CD90⁺



CD90-FITC

Lack expression



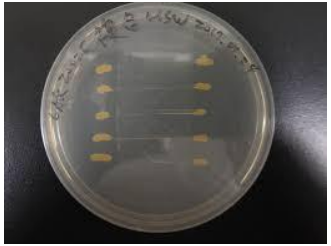
IgG1 isotype control　　CD34-PE

CD34⁻

項目	改良分離法	傳統分離法
分離環境	類GMP、GTP製程	實驗室製程
分離所需時間	兩小時內	約六小時
產量	10^7/g	5x10^7/g
所需放大時間	三天	七天

嚴格品管控制

1. Mycoplasma detection
2. Pyrogen test
3. Sterility test



脂肪間質幹細胞檢測報告

O'Pet 萬寶特生醫

附件一

萬寶特生醫股份有限公司
O'Pet Biomedical Co., Ltd.

自體細胞生物技術服務品管書

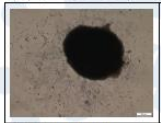
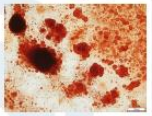
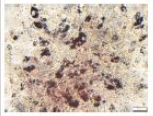
- 動物醫院(Animal hospital)或獸醫診所(Veterinary clinic):_____
- 獸醫師姓名(Name of Veterinary):_____
- 動物別(Animal type)：狗 (Canine) / 貓 (Feline)
- 寵物名(Pet's Name):_____
- 動物品種(Breed)：_____
- 收案日期(Case collection)：____年___月___日 (yyyy/m/d)
- 動物年齡(Age)：____歲
- 體重(Weight)：_____Kg
- 性別(Male/Female)：_____
- 細胞來源：自體脂肪間質幹細胞
- 分析內容：

(一) 幹細胞型態 (ADSCs image)

左圖為範例圖

細胞代數(Cell passage number)：_____代

(二) 分化能力測試 (Differentiation ability test)

		
軟骨分化 Chondrogenesis	硬骨分化 Osteogenesis	脂肪分化 Adipogenesis
正常 Normal	正常 Normal	正常 Normal

上圖皆為範例圖

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(三) 無菌測試 (Sterility Test)

Negative

(四) 內毒素測試 (Endotoxin Test)

Negative，依據美國食品藥物局(FDA)之規定，非經腸道給予的產品，建議內毒素上限為 5 EU/公斤，符合注射液標準。

- 結論：此分析報告顯示脂肪間質幹細胞型態正常，採樣/分離及培養過程中無其他病原污染，且經無菌測試與內毒素測試皆為陰性。經幹細胞分化能力測試後，證實該細胞持有脂肪間質幹細胞之三重分化能力(軟骨、硬骨及脂肪細胞)。經細胞標記檢視鑑定符合脂肪間質幹細胞特性。這些脂肪幹細胞只適用於動物細胞治療。

(Conclusion: The report shows that the morphology of ADSC is normal and pathogen free during the adipose tissue collection and isolation. It is also approved that the Endotoxin & Sterility Tests are all negative as cell culture processing procedures. After the differentiation assay, the cells are identified with triple differentiation assay which are qualified with cartilage, bone, and adipose cells. Also, the CD markers that present on ADSC. These cells are for animal cell therapy only.)

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O'Pet
萬寶特生醫

Thank you for listening

專業　創新　守護寵物健康

COAST

名稱:

評估日期:

第一步驟 : 犬隻評級

飼主觀察狀況 居所/非門診環境		**獸醫師評估** 獸醫門診			
CMI (CBPI or HCPI 擇一)	**犬隻不適程度**	**對姿勢之影響 (靜止時)**	額外敘述	**對移動之影響**	額外敘述
0 或 非常低分 或 “臨床上不受影響” ☐	無 ☐	正常 ☐	靜止時姿勢適合該品種 適當的肢體負重 前後肢間的體重分佈適當	正常 ☐	對稱 適當的承重 適當的體重分佈 步態流暢
低分 或 “輕度受影響” ☐	低度 ☐	輕度異常 ☐	肢體負重出現輕微異常 靜止時的體重分佈有輕微改變	輕度異常 (輕微的改變) ☐	可能以某種步態或從事某種活動，運動會受到輕微影響 輕微步態僵直 體重分布有輕微改變 輕微不對稱 輕微跛行 起身無困難 (站起)
中等分數 或 “中度受影響” ☐	中度 ☐	中度異常 ☐	肢體負重出現明顯異常 靜止時的體重分佈有明顯改變	中度異常 (明顯的改變) ☐	在所有步態和活動中，均出現一致的運動異常 明顯的步態僵直 體重分部有明顯的改變 明顯地減少患肢的使用 站立期明顯的減短 起身有些困難 (站起)
高分 或 “重度受影響” ☐	無法忍受 ☐	重度異常 ☐	站立時感到焦躁不安 不願意(困難) 維持站立 靜止時的體重分佈有重度改變 肢體負重重度異常	重度異常 (非常明顯的改變) ☐	掙扎著移動 / 不願意移動 經常出現跛行 重度的重心轉移 起身明顯困難 (站起)

COAST

名稱：

評估日期：

第一步驟：關節評級

獸醫師評估 獸醫師門診				
操作時疼痛感	**被動性活動程度**	額外敘述	**X光檢查**	額外敘述
無 ☐	正常 ☐	正常	經X光檢查無骨關節炎的徵象 ☐	如果臨床前即處於“有風險的狀態”，X光檢查或將證實該犬隻可能具有如：發育不良和/或外傷等風險因子
輕度 ☐	輕度異常 ☐	關節活動範圍輕微減少 無關節輾軋音 輕微關節增厚	輕度異常 (輕微的改變) ☐	骨關節炎的早期徵象 極少的骨刺
中度 ☐	中度異常 ☐	關節活動範圍明顯減少 肌肉萎縮 明顯關節增厚	中度異常 (明顯改變) ☐	明顯的骨刺
重度 ☐	重度異常 ☐	極其有限的關節活動範圍 關節輾軋音 嚴重肌肉萎縮 重度關節增厚 觸診時發現解剖結構異常或結構錯位	重度異常 (非常明顯改變) ☐	大量骨刺增生 或關節變形

對所有受影響的關節重複這份評估(工作表提供如下)

COAST

名稱 :

評估日期 :

第二步驟 :所有受影響關節的評級

關節右側	操作時疼痛感	體檢	X光檢查
手部關節			
腕關節			
肘關節			
肩關節			
Pes			
踝關節			
膝關節			
髖關節			

關節左側	操作時疼痛感	體檢	X光檢查
手部關節			
腕關節			
肘關節			
肩關節			
Pes			
踝關節			
膝關節			
髖關節			

COAST

名稱：

評估日期：

第三步驟: 綜合評級分數做為總體嚴重性的分數和階段

	犬隻評分					關節評分				總體嚴重程度 (最高分)
	CMI 評估表	不適感	姿勢	運動		操作時疼痛感	體檢	X光檢查		
低度/無	☐	☐	☐	☐		☐	☐	☐		
輕度	☐	☐	☐	☐		☐	☐	☐		
中度	☐	☐	☐	☐		☐	☐	☐		
重度	☐	☐	☐	☐		☐	☐	☐		

不太嚴重

非常嚴重

總體嚴重程度	描述		OA的階段
	臨床前	無明顯危險因子	0
		“危急狀態”：至少有一個導致可見骨關節炎的因素 例如：品種素因、關節損傷、肥胖、劇烈活動和/或不正常增生或關節外傷的X光徵象	1
	輕度		2
	中度		3
	重度		4