Novel Veterinary Treatments for Arthritis: New Products Containing *Adult* Canine Stem Cells.

Drs Angus Ross and Jamie Geddes are principals at the Ku-Ring-Gai Veterinary Hospital (KVH) in Sydney. For the past 18 months, they have been working closely with both Regeneus Pty Ltd, an Animal Health company, as well as with members of the Science Faculty of the University of Technology Sydney (UTS) to develop new non-drug based technologies for the therapeutic amelioration of arthritis in dogs. These interactions have resulted in an adult stem cell-containing product called AdiCell™.

They have thus pioneered the use of autologous stem cell therapy in small animals in Australia and have developed strict procedures for the AdiCell process that incorporates comprehensive arthritis assessment, tissue harvesting and re-implantation protocols.

Adult stem cell-containing products are thought to have therapeutic uses that extend far beyond arthritis. Jamie is evaluating the potential clinical applications of AdiCell™ for the treatment of neoplastic, ocular and organ based diseases and has developed liposuction methodology for the isolation of adult stem cell-containing cell treatments. In addition, with the assistance of Fresenius Medical Care (Asia-Pacific), Angus is evaluating clinical applications in nephrology and has also established the first haemodialysis unit for pets in Australia. Angus and Jamie also operate a diagnostic imaging service (CT, Ultrasound and Endoscopy) for other veterinarians on a vet-to-vet only basis.

Rebecca Webster, a former senior KVH surgical nurse, is working with Associate Professor Ben Herbert on the effects of adult mesenchymal stem cell-containing cell populations on arthritic joints in animals as part of a Masters degree at UTS. She has now joined Regeneus full-time and her work includes the use of the GAITRite pressure plate system for the analysis of limb movement and coordination.

World-wide media reports about stem cell therapies are becoming commonplace as stem cell applications are being pursued in diverse areas including cardiology, orthopaedics, oncology and internal medicine. Owing to the differences in national regulatory frameworks, stem cell technologies vary greatly in regards to the species of application, the degree of research involved and the clinical applications. In this article we deal only with *adult* stem cell populations and their derivatives which are found in various tissues throughout the mammalian body. We *do not use* or review embryonic stem cells, which are associated with a host of clinical, regulatory and ethical problems. We also emphasize the use and advantages of *autologous* cell populations which have not been expanded in artificial tissue culture conditions that can cause extensive remodelling of cell fate.

A collaborative effort between Regeneus Pty Ltd, Ku-Ring-Gai Veterinary Hospital and members of the UTS Science Faculty has resulted in a commercial application which contains adult mesenchymal stem cells and associated cell populations derived from fatty tissue. This procedure, designated AdiCell™, is being utilised to treat osteoarthritis in dogs and is already yielding very promising results. To date a small number of cats have been treated for osteoarthritis however positive results are not as yet replicable due to the small sample size.

1. What patients are suitable for treatment with AdiCell™?

Sixty dogs have been treated to date and all cases were patients of KVH with owners receiving full disclosure and giving informed consent. Patients treated fit into 3 general categories.

- Elderly arthritics - dogs that have the more classical signs of degenerative joint disease (DJD) from years of wear and tear, including osteoarthritis of the hip, stifle, carpus, shoulder, elbow and tarsus. Such dogs are mostly over eight years of age.
- Premature arthritics - dogs who in the past have suffered an orthopaedic or arthrological event, including developmental orthopaedic diseases such as a fragmented coronoid process and hip

dysplasia, intra-articular fractures and ruptured cruciate ligaments. These patients have thus developed premature onset of DJD.

- The remainder of cases include primary hip dysplasia and immune-mediated arthropathy cases.

In almost all cases, traditional diagnostic and therapeutic approaches had already been made in regards to osteoarthritis, including radiographs, and in certain cases joint fluid assessment. This was followed by dietary and exercise recommendations, physiotherapy, pharmaceutical intervention (non-steroidal anti-inflammatory drugs (NSAIDs) and chondroprotective agents), neutraceuticals and alternative therapies.

Patient selection was initially based on the diminished capacity of conventional therapies to provide good symptomatic control. Furthermore, a key area in patient selection involves the identification of comorbidities to determine how they impact on the success of the AdiCell™ process.

2. Assessment

Much emphasis has been placed on the initial assessment process in order to obtain as much pre-treatment data as possible and to ensure that all osteoarthritic joints are appropriately identified. A strict assessment protocol has been applied for assessment including:

Photo 1: Preparation for CT scanning of elbows

- A complete history, physical and musculoskeletal examination.
- Neurological examination.
- Orthopaedic examination, subjective gait analysis and scoring (numeric rating scoring).
- Full blood and urine testing including CK, T4, ANA and RF antibody testing.
- Diagnostic imaging including radiographs, ultrasound and CT scanning. CT scanning helps to reduce imaging time and can significantly increase

joint detail enabling exclusion of pre-existing surgical joint lesions, especially in elbows (Gielen et al 2009).

- Joint fluid cytology and culture and susceptibility testing, or synovial biopsy where indicated.
- Objective Gait analysis

3. GAITRite pressure analysis

Regeneus and KVH are using a sophisticated pressure analysis system, known as GAITRite, to analyse the gait of dogs before and after AdiCell™ treatment. GAITRite analysis allows the measurement of stride length, stance time, symmetry and peak vertical pressure for each limb.

This system was originally developed to measure and record temporal and spatial parameters of human gait and has been adapted for gait analysis in dogs. The system comprises a 4 metre walkway with grids of 16,128 embedded pressure-sensitive sensors connected to a computer. Cameras are connected to the system, enabling simultaneous video- imaging.

Dogs are walked on a loose leash up and down the mat at a range of speeds. Pressure readings occur from each paw fall and video data is also recorded. Ten walks are recorded at a session and the data are then analysed together with the video images. Pressure, spatial and time data are recorded and these generate substantial amounts of raw data, including pressures for each paw fall, the pressure profile across each paw, the centre of pressure, the stance time, stride time, stride length and velocity.

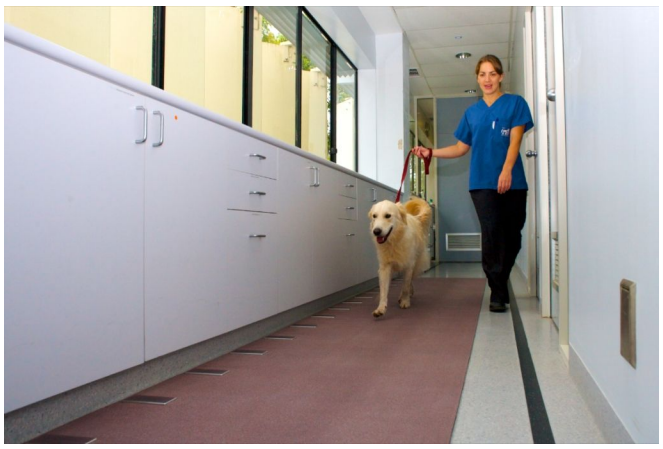

Photo 2: GAITRite pressure testing

One of the advantages of this set-up is the ability to capture sequential steps, to measure multiple gait cycles with one pass or walk. More traditional force plate systems are short and only allow a single gait cycle to be captured. By capturing multiple gait cycles, symmetry comparisons can be performed and this is where the GAITRite system is invaluable. In a healthy dog for example, the ratios between the left paws and the right paws should be close to 1, whereas a dog that is lame in one limb will have a skewed left to right ratio. Similarly the hind to fore ratio will also be affected.

Paw Parameters	L. Hind	R.Hind	L.Front	R. Front
Cycle Time (sec)	.431	.43	.434	.435
Stride Length (cm)	97.59	95.69	98.14	97.29
Swing %	62.4	64.4	55.5	54.3
Stance %	37.4	35.6	44.2	45.7
Number Sensors	11.5	11.0	14.3	15.8
Peak Pressure	29.3	30.8	39.0	56.3
Mean Pressure	2.5	2.8	2.7	3.6

Parameters	
Distance (cm)	294.0
Ambulation Time (sec)	1.30
Velocity (cm/sec)	226.2
Complete Cycle (First paw repeats)	
Number of Contacts	16
Cadence (first paw repeats)	184.6
Cycle Time Differential F/B(sec)	.00
Stride Length Differential (cm)	2.65
Cycle Time Differential L/R(sec)	.00

Data table 1: GAITRite pressure analysis, paw placements from gait cycle with dog walking from right of graph to left and the raw data information obtained.

4. Adipose tissue

Adipose tissue is removed in the operating theatre under general anaesthetic and is performed either by excision of the inguinal fat pad or via liposuction. Once adipose tissue is harvested, joints requiring implantation are clipped and the patient is removed from general anaesthetic and given further levels of opiate as required.

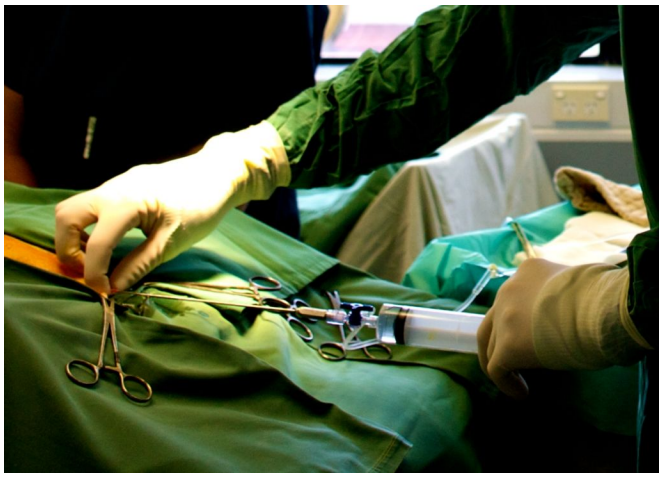

Photo 3: Liposuction harvesting of adipose tissue

5. Isolation of an adult stem cell-containing cell population

Adipose tissue is processed using a procedure that takes less than two hours. The process routinely results in a small volume and contains between one and fifty million cells (depending on the patient). The cell population contains a mixture of various cell types including adult mesenchymal stem cells and their derivatives. A patent application has been filed on the procedure.

6. Transplantation of autologous cells

The patient is lightly re-anaesthetised, generally via Isofluorane induction, and the joints are surgically prepared. Transplantation is performed via the standard technique of arthrocentesis for each joint. Joint fluid is aspirated to ensure correct placement before the cells are injected and the joint fluid is submitted for laboratory analysis. Up to 12 joints have been treated in the one procedure.

7. Post Operative Conditions

Patients remain in hospital over night on a methadone (Methone, Parnell) constant rate infusion, with appropriate antimicrobial prophylaxis and NSAIDs. They are discharged the following day on NSAIDs and anti-microbials, if required. Patients are generally reviewed at 3 and 10 days post-procedure and then at monthly intervals.

8. Assessment of AdiCell treated dogs

Thirty-three dogs that received Adicell™ treatment for arthritic joints had a scored veterinary assessment prior to treatment and at 10 days and monthly intervals for 6 months and then at 3 monthly intervals on an ongoing basis. The examination involved numeric rating scoring (NRS) for lameness, range of motion (using goniometry), functional disability, pain on manipulation, proprioception, crepitus, swelling, ability to climb stairs and jump. The scoring system was based on that of Hielm-Björkman (2007) and covered a range from 0 to 4, with 0 being the best possible score and 4 being the worst. For example, lameness was scored as follows;

0 = not detectable
1 = intermittent
2 = persistent
3 = ambulatory with assistance
4 = non- ambulatory

Treated animals were also assessed by owners completing a questionnaire that involved 20 questions relating to mobility on a visual analogue scale (VAS). These questions are designed as a 100 mm VAS with one hundred being the best possible score and zero being the worst. (Hudson et al. 2004)

Within 10 days of being treated with AdiCell™, some arthritic dogs showed a marked improvement in mobility. There was a significant difference in scoring in both the veterinary examinations and in the owner-completed questionnaires. Statistical analysis, using single one-way ANOVA, showed that monthly assessments for both physical examinations and owner questionnaires are significantly different ($p<0.05$) to pre-treatment scores.

Case study 1: Tully, four-year old Border Collie.

Tully is a champion agility dog that had been suffering from early stage osteoarthritis in the stifles and has been intermittently symptomatic for four months. A routine diagnostic approach was performed and failed to reveal any other condition beyond stifle DJD. He had received four-week courses of pentosan polysulphate (Cartrophen, Naturevet) and was on daily doses of firocoxib (Previcox, Merial). Significant complications ensued with gastric and renal side effects and the owner elected to try AdiCell™ therapy.

Photo 4: Tully, four year old Border Collie

The NRS assessment for Tully prior to Adicell treatment was an average score of 1.9. At Tully's one month assessment the average score was reduced to 0.1. **He is now at six months and is still at a score of 0.1 with all medication having been withdrawn.**

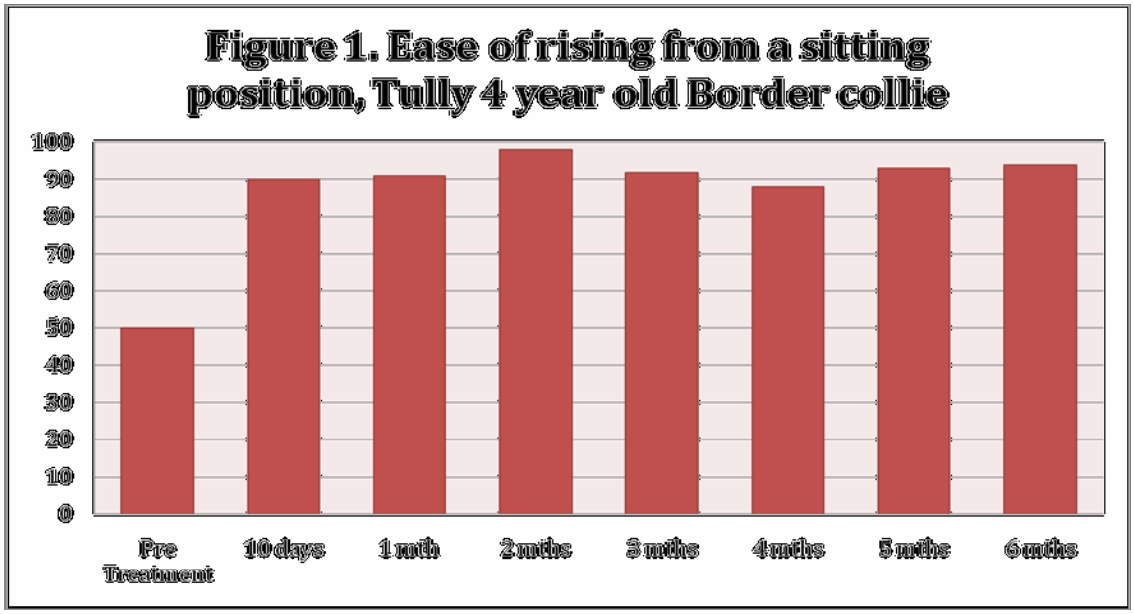

Figure 1 shows an example of one of the twenty questions which owners are asked regarding the assessment of their animals. "Rate your dogs ease in rising from a sitting position". These questions are designed as a 100 mm VAS with one hundred being the best possible score and zero being the worst. (Hudson et al. 2004).

Case study 2: Sassy, 13 year old German Shepherd

Sassy has severe osteoarthritis in both stifles and hip joints. She had undergone unilateral hip replacement and bilateral cranial cruciate ligament repair surgery by age nine. Sassy was receiving carprofen (Rimadyl, Pfizer), tramadol (Tramal, CSL) and diazepam (Valium, Roche) daily to manage pain. In June 2008 both stifles and right hip were treated with Adicell™.

Sassy's recovery has been more protracted than other dogs. The pre-treatment NRS assessment score averaged 2.9 (Table 1). At 10 days post treatment the score averaged 2.1. By three months her score averaged 1.5 and at six months she had improved noticeably to an averaged score of 1.1.

Sassy's medication has been reduced by more than half since receiving Adicell™ treatment. Sassy's owner videoed Sassy at weekly intervals to document the effect of the Adicell treatment. **The video can be viewed at http://www.youtube.com/watch?v=NtEdON8OhZ0**

	Pre-treatment	10 days	3 months	6 months
Lame at walk	2.0	2.0	1.0	1.0
Lame at trot	2.0	2.0	1.0	1.0
Lame at Run	2.0	2.0	1.0	1.0
Range of motion	4.0	3.0	1.0	2.0
Functional disability	3.0	2.0	2.0	1.0
Crepitus	3.0	2.0	3.0	1.0
Swelling	2.0	0.0	0.0	0.0
Jumping	4.0	4.0	4.0	3.0
Stairs	4.0	2.0	2.0	1.5
Proprioception	2.0	0.0	0.0	0.0
Pain on manipulation	4.0	2.0	1.0	0.5
Average	**2.9**	**1.9**	**1.5**	**1.1**

Table 1: Veterinary NRS examination scores for Sassy, with 0 being the best possible score and 4 being the worst score.

9. The nature of improvements brought about by the AdiCellTM procedure

Most dogs show the greatest improvement within three months, although a proportion of dogs have continued to improve at a steady rate for up to 11 months, which represents the duration of the study. At this time, no cases where dogs have shown improvement have shown subsequent deterioration in NRS assessment scores except for cases that developed co-morbidities.

There are a number of possible explanations as to why AdiCell appears to work more quickly than anticipated intuitively:

- Molecules produced by the transplanted cells leading to anti-inflammatory effects and pain modulation.

- Stimulation of the local environment to generate new cartilage via the transplanted cell population and/or the resident cells of the synovial sac.
- Physical lubrication of the joint via the transplanted molecules.

Production of extracellular molecules. Recent results from Regeneus' research at UTS have demonstrated that *adult* adipose-derived cell populations secrete proteins known to have powerful potentially anti-inflammatory capabilities in other model systems. Using sophisticated Mass Spectrophotometric technologies, Associate Professor Ben Herbert and Ph.D. student Michael Medynskyj have identified a cocktail of proteins known from published studies to down regulate various stress markers in cells around them.

Production of new cell populations. Recent molecular breakthroughs in permanent cell labelling technologies now allow for the unambiguous tracking of cells by their intrinsic production of a protein, termed Green Fluorescent Protein (GFP). Such auto fluorescing cells can be transplanted to any tissue or organ of the body and their subsequent behaviour, division and differentiation, as well as their migration to other sites within the body, can be followed with ease. When GFP-labelled adult mesenchymal stem cells were transplanted into the damaged joints of goats, cells were observed to embed into the surrounding tissue and to generate new cartilage (Murphy, et al. 2003). Studies in rabbits have also shown that new cartilage growth occurs as quickly as two weeks post-treatment (Wakitani, Goto et al. 1994) At this stage, further research is required to understand the underlying mechanisms involved in the clinical improvements cited above.

10. Results from other adult stem cell-based therapy in dogs with osteoarthritis

Two recent studies have examined the effect of adipose-derived adult mesenchymal stem cell- containing populations in the treatment of osteoarthritis in dogs. A double-blinded trial of 21 dogs with osteoarthritis of the hip joint was reported by Black, et al. (2007). The study used similar scoring methods to those described here. Dogs treated with cells showed a

significant improvement in lameness and functional ability compared to the dogs that received the placebo.

A similar study of canine osteoarthritis in the elbow joint was conducted in 2008 and demonstrated an improvement in lameness, functional disability and stiffness scores following a single injection of adipose-derived adult mesenchymal stem cell-containing populations into the joint (Black et al. 2008). Results improved over time post-injection, with significant improvement still reported at 180 days post treatment.

Condition	adipose derived cells	Number of cells	Reintroduction	Results	Reference
Canine hip osteoarthritis	Autologous not cultured	4.2-5 x 10^6	Intra-articular	Statistically significant improvement in lameness	(Black, et al. 2007)
Canine elbow osteoarthritis	Autologous not cultured	3-5 x 10^6	Intra-articular	Statistically significant improvement in examination scores for lameness, joint stiffness and functional disability	(Black, et al. 2008)

11. Discussion

Beyond the risks of anaesthesia, fat harvest site bruising and irritation, as well as the risk of infection in the harvest site or implantation site, the AdiCell™ procedure is well tolerated. Owners are generally surprised by the speed of recovery. If a surgical fat pad excision is required, the harvest wound may be bruised, although this rarely worries the patient.

Of the 33 cases assessed, 3 dogs showed no signs of improvement. Most importantly, on the veterinary and client qualitative assessment data to date, *no cases have been worsened by the process*.

At present, work is limited by the timeframe, limitations of NRS and VAS (Waxman et al. 2008) and degree of quantitative and cellular data available. There is a need for further objective studies with MRI and nuclear medicine currently being considered blinded and comparison studies.

However, for osteoarthritic patients who are receiving diminishing benefits from their current therapeutic regime, the AdiCell™ process represents a significant advancement. **Critically, improvements occur in pain control, range of motion, mobility and activity.** As a generalisation, doses of NSAIDs can be lowered substantially, but in elderly patients they are usually not withdrawn. For the younger animals who have suffered premature degenerative joint disease, improvements have so far been sustained.

At present the duration of benefit following treatment with AdiCell is not known, but the effects would appear to last at least 9 months. Further research needs to be performed to ascertain the benefits of utilising AdiCell™ earlier in the osteoarthritis process to determine if regenerative techniques can delay or prevent the onset of osteoarthritis as well as treating it.

12. Conclusions

The use of adult stem cell-based therapies in animals, although still in its infancy, is already producing successful outcomes. This autologous approach using an animal's own cells for therapeutic purposes appears to be safe and well tolerated. Importantly, unlike most approaches with stem cells which use growth in artificial tissue culture conditions to expand the cell population prior to transplanting them to a patient, the cells used in AdiCell by Regeneus are not expanded in tissue culture. They are rapidly processed and returned very quickly to the patient and hence avoid the myriad of problems that arises from *ex vivo* culture. The future of adult stem cell-based therapies for animals is certainly very promising.

13. References

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