

STEM CELLS



FourLegRehabInc
CANINE REHAB EDUCATIONAL RESOURCES



Introduction

To follow up on the information about PRP, I wanted to hunt down research pertaining to Stem Cells as well.

Because what is most readily available in clinical small animal veterinary medicine practice is adipose-derived stem cells (or 'autologous stromal cells'), I chose to narrow my search and

reporting to this kind of stem cell. Furthermore, I limited the search to tendon and joint. I'll have to follow up another time with spinal cord and nerve... as I know there is some interesting research in that direction. Anyways, I'm hoping you find the information to be as interesting as I did!

I was surprised at the research I did find, yet also surprised at the lack of research in this area. Bottom line, there is promise in this field, it's just still in its infancy is all!

Cheers,
Laurie

WHAT IS A STEM CELL?

The best definition I have ever read or heard came from a staff member with dual degrees in biology and anthropology. "A stem cell is an undifferentiated cell", she said. (I stared at her blankly and replied, "That means nothing to me."). An undifferentiated cell has not

yet determined what it wants to do or be. So it can become anything! Put it into a tendon, it can become a tenocyte. Put it into cartilage, it can become a chondrocyte. Put it into bone and it can become an osteocyte. So the thought is that if you put it into injured tissue, it can create the tissue needed to heal that structure. "Ah, now I get it!"

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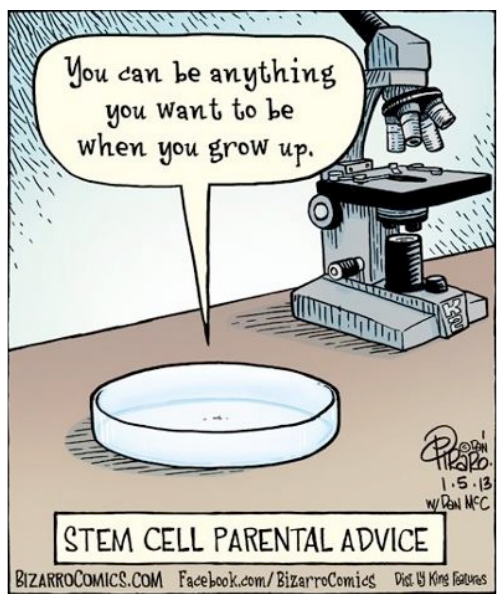
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Mesenchymal Stem Cells Background

Mesenchymal stem cells (MSC) have been derived from bone marrow, umbilical cord blood, and adipose tissue.

- Harvesting bone marrow is an invasive procedure.
- Differentiation potential, & maximal lifespan of Bone Marrow-derived MSC declines with increasing age.
- Adipose tissue can be harvested in a less-invasive way & can yield larger volumes of MSC.

Kern S. et al. 2006. Comparative analysis of mesenchymal stem cells from bone marrow, umbilical cord blood, or adipose tissue. Stem Cells 24: 1294-1301.



Lessons from Pathology: Why would we think fat has any stem cells?

1

ECTOPIC BONE HAS BEEN FOUND IN THE SUBCUTANEOUS FAT IN CHILDREN WITH PROGRESSIVE OSSEOUS HETEROPLASIA. THIS FINDING IMPLIES THAT ADIPOSE DERIVED STEM CELLS ARE 'TRIPOTENT', WITH THE CAPABILITY OF ADIPOGENIC, CHONDROGENIC, AND OSTEOGENIC DIFFERENTIATION POTENTIAL.

2

A STEM CELL POPULATION WITHIN ADIPOSE TISSUE IS RESPONSIBLE FOR REPLACING MATURE ADIPOCYTES THROUGH THE LIFETIME OF THE INDIVIDUAL. FOR EXAMPLE, THE REMOVAL OF A FAT PAD IN RATS SIGNALS THE GENERATION OF NEW ADIPOSE TISSUE. THIS OCCURS NOT ONLY THROUGH AN INCREASE IN THE VOLUME OF PRE-EXISTING ADIPOCYTES BUT ALSO THROUGH THE GENERATION OF NEW ADIPOCYTES FROM A PROGENITOR OR STEM CELL POOL.

3

LIPOMAS AND LIPOSARCOMAS ARE THE MOST COMMON DIAGNOSES OF SOFT TISSUE TUMORS PRESENTING IN A CLINICAL SETTING. OFTEN, THESE TUMORS OCCUR IN SUBCUTANEOUS OR VISCERAL ADIPOSE DEPOTS AND PROLIFERATE SLOWLY. THE LIPID CONTENT OF BENIGN LIPOMAS AND WELL DIFFERENTIATED LIPOSARCOMAS IS COMPARABLE TO THAT SEEN IN NORMAL ADIPOSE TISSUE.

Background & Science Continued...

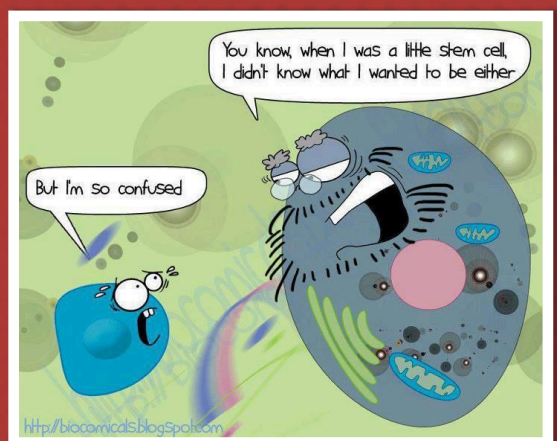
Cell proliferation?

- In vitro, adipose-derived stem cells (ASCs) display a cell doubling time of 2 to 4 days, depending on the culture medium and passage number.
- In vitro studies have shown the likely for stem cell therapies to provide tendon regeneration rather than repair of tendon tissue. Regeneration involves slow replacement of tissues with identical tissue. It occurs readily in the embryo, hardly at all in the neonates and is never observed in adults. In contrast, repair is a more rapid process, involving the inflammatory cell cascade, followed by matrix deposition and then a remodeling process, which attempts, in part, to regenerate damaged tissue in the adult.

Potential utility?

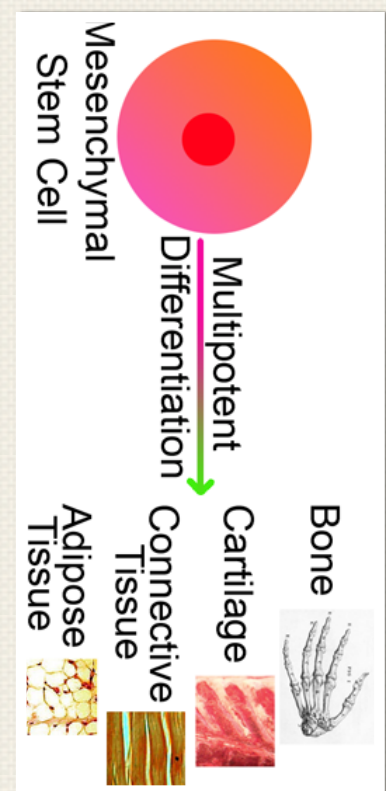
- ASCs delivered into an injured or diseased tissue may secrete cytokines & growth factors.
- ASCs stimulate the recruitment of endogenous stem cells to the site and promote their differentiation (i.e. so they become cells that will create the tissues in which they live).
- ASCs might provide antioxidants, chemicals, free radical scavengers, and chaperone/heat shock proteins at an ischemic site. As a result, toxic substances released into the local environment would be removed, thereby promoting recovery of the surviving cells
- MSCs can deliver new mitochondria to damaged cells, thereby rescuing aerobic metabolism.

**STEM CELL RESEARCH CAN REVOLUTIONIZE MEDICINE,
MORE THAN ANYTHING SINCE ANTIBIOTICS
- RONALD REAGAN**



RESOURCE:

Gimble JM et al. 2007. Adipose-derived stem cells for regenerative medicine. *Circulation Res.* 100: 1249-1260.



FOR THE RECORD

We are talking about adult stem cells, not embryonic stem cells.

Benefits being:

- There is no chance of immune reaction if the cells are harvested from the same patient.
- They are non-tumorigenic.
- They are multipotent (i.e. they can become many different types of cells - not any or all types... but many different types)
- They are much easier to come by!

Tendons...

Carvalho A, et al. 2013. Equine tendonitis therapy using mesenchymal stem cells and platelet concentrates: a randomized controlled trial. 4(4): 85

A lesion was created in the SDFT in 8 horses. Two weeks later, the lesions were treated with adipose-derived mesenchymal stem cells. At 16 weeks post-therapy a biopsy was taken.

The main results observed from the histopathological evaluation of the treated group were as follows: a prevention of the progression of the lesion, a greater organization of collagen fibers, and a decreased inflammatory infiltrate. A lack of progression of the lesion area and its percentage was observed in the ultrasound image, and increased blood flow was measured by Power Doppler.

Beitzel, K., et al. 2013. The future role of mesenchymal stem cells in the management of shoulder disorders. Arthroscopy 29(10): 1702-1711.

This systematic review found 17 in vivo and in vitro human and animal studies. The current literature regarding therapeutic use of MSCs in shoulder surgery is limited. Although in vivo animal studies have shown some promising approaches to enhance tendon-to-bone healing, the use of

MSCs for tendon shoulder surgery should still be regarded as an experimental technique.

Valencia, M., et al., 2014. Application of adipose tissue-derived stem cells in a rat rotator cuff repair model. Injury 45 Suppl 4: S22 - 27.

Healing tissue of the rotator cuff does not regenerate the native enthesis (where the tendon attached to the bone); fibrovascular scar tissue is formed instead and this has less favourable biomechanical properties. The purpose of this study was to determine if the application of adipose tissue-derived stem cells (ASCs) could improve biomechanical and histological properties of the repair.

This study used 100 rats randomly allocated into 2 experimental groups.

The application of ASCs in a rat rotator cuff repair model did not improve the biomechanical properties of the tendon-to-bone healing. However, the ASCs group showed less inflammation, which may lead to a more elastic repair and less scarred healing.

Pacini S, et al., 2007. Suspension of bone marrow-derived undifferentiated mesenchymal stromal cells for repair of superficial digital flexor tendon in

race horses. Tissue Eng. 13:2949-2955.

Del Bue, M., et al., 2008. Equine adipose-tissue derived mesenchymal stem cells and platelet concentrates: their association in vitro and in vivo. Vet Res Commun. 32 Suppl 1:S51-5.

Pacini et al (2007) showed recovering of normal activity in horses affected by superficial digital flexor tendinopathy managed with targeted intralesional injection of BMSCs. Also adipose derived stem cells were showed to be effective in the treatment of equine tendinopathies leading to normal horse activity recovery (DelBue et al, 2008).

CONCLUSION

As demonstrated by these preliminary studies, management of tendinopathies with stem cells is promising even though more clinical studies are needed to validate this treatment approach.

One interesting study looked at whether injection of a steroid drug (betamethasone or methylprednisolone) had an impact on adipose-derived MSCs. Essentially, at concentrations of 0.01 mg/mL and 0.1 mg/mL (respectively) did not impact the MSC's and may have had a beneficial effect on cellular activity. However, at concentrations of 1 mg/mL, there was a cytotoxic effect.

Marycz K, et al. 2014. Effects of steroids on the morphology and proliferation of canine and equine mesenchymal stem cells of adipose origin – in vitro research. Acta Vet Hung 62(3): 317-333.

Joint & Cartilage...

Well, it was harder than one would think to get papers that looked at joint & cartilage and adipose-derived stem cells. I found more from bone marrow, but that wasn't the point of my search. One review paper (Filardo et al 2013) reported:

“The systematic research showed an increasing number of published studies on this topic over time and identified 72 preclinical papers and 18 clinical trials. Among the 18 clinical trials identified focusing on cartilage regeneration, none were randomized, five were comparative, six were case series, and seven were case reports; two concerned the use of adipose-derived MSCs, five the use of BMC, and 11 the use of bone marrow-derived MSCs, with preliminary interesting findings ranging from focal chondral defects to articular osteoarthritis degeneration.”

It further concluded:

“Despite the growing interest in this biological approach for cartilage regeneration, knowledge on this topic is still preliminary, as shown by the prevalence of preclinical studies and the presence of low-quality clinical studies. Many aspects have to be

optimized, and randomized controlled trials are needed to support the potential of this biological treatment for cartilage repair and to evaluate advantages and disadvantages with respect to the available treatments.”

Filardo, G., et al., 2013. Mesenchymal stem cells for the treatment of cartilage lesions: from preclinical findings to clinical applications in orthopaedics. *Knee Surg Sports Traumatol Arthosc* 21(8): 1717-1729.

I subsequently searched for adipose-derived MSCs subsequent to that 2013 paper, and I did find some promising studies.

Jo C. et al. 2014. Intra-articular injection of mesenchymal stem cells for the treatment of osteoarthritis of the knee: A proof-of-concept clinical trial. *Stem Cells* 32: 1254-1266.

This paper just injected stem cells of different concentrations into the knee joint. Previous studies had implanted them. The stem cell injection protocol that worked the best was a single injection of a 'high dose' of stem cell concentrate (1×10^8 cells) in 3mL of saline solution. (The low dose group consisted of 1×10^7 , and medium dose group received 5×10^7). As such, it would appear that the concentration of stem cells was most important.

What results were found at the 6-month follow up mark?

- The WOMAC Index (as self administered functional test) was used to determine functional outcomes, as was a pain score, and Knee Society Clinical Rating System (KSS):
 - All ratings improved in the high-dose group.
 - Whereas only the KSS improved for the low-dose group.
- Radiological outcomes were assessed:
 - No changes were noted on x-ray in any treatment group.
- MRI was used to look at the regenerated cartilage:
 - The size of cartilage defect measured with MRI significantly decreased in all joint surfaces measured (except the patella) in the high-dose group.
 - No defect changes were noted in the low- or medium-dose groups.
 - Cartilage volume was also improved in all groups at the 3-month stage, but at 6-months, only

Con't...

- the high-dose group showed improvements at the medial femoral & tibial condyles (not at the other surfaces however).
- Arthroscopy evaluated changes in cartilage defects at the 6-month mark:
 - Macroscopically, cartilage regeneration was seen in the most severely degenerated areas.
 - Depth of cartilage defects appeared reduced as measured by a calibrated probe in the high dose-group (improvement was seen in all areas except the lateral femoral and tibial condyles & patella).
 - NOTE: The study did not mention the look of the cartilage in the low or medium-dose groups!
- Histological Outcomes:
 - Generally, pre-injection, there was no articular cartilage on the medial femoral

condyles. After injection, at the 6-month mark... there was evidence of cartilage, with indication that maturation of the cartilage was still in progress.

And then there 's this one:
Koh, Y. G., et al. 2015. Clinical results and second-look arthroscopic findings after treatment with adipose-derived stem cells for knee osteoarthritis. 23(5): 1308 - 1316.

They took 30 people over the age of 65 with knee OA. They obtained adipose tissue from both buttocks by liposuction, and 'did what they did' to get a mean of 4.04×10^6 stem cells. They injected the prepared solution into the knees and followed up at 3 months, 12 months, and 2 years. All patients were evaluated using knee injury scoring systems, pain scales, and radiographic evaluation, and 16 underwent a second look arthroscopy.

- All clinical results (knee function scoring systems & pain scales) improved at the 2 year follow up compared to the 12-month follow up.
- 5/30 had a worsening grade radiographically.
- Of those that underwent the second arthroscopy, 14/16 (87.5%)

improved or maintained cartilage status.

(Sign me up for this study... liposuction AND better knees!!!)

Manferdini C., et al. 2013. Adipose-derived mesenchymal stem cells exert anti-inflammatory effects on chondrocytes and synoviocytes from osteoarthritis patients through prostaglandin E2. Arthritis Rheum 65(5): 1271-1281.

- The anti-inflammatory effects of AD-MSCs are dependent on the inflammatory status of OA chondrocytes and synoviocytes. AD-MSCs seem to be able to sense and respond to the local environment.



STEM CELLS FOR OA IN DOGS, CLINICALLY...

... (this is all!)

- (for HIPS): Dogs treated with adipose-derived stem cell therapy had significantly improved scores for lameness and the compiled scores for lameness, pain, and range of motion compared with control dogs.
- (for ELBOWS): Veterinarians assessed each dog for lameness, pain on manipulation, range of motion, and functional disability using a numeric rating scale at baseline and specified intervals up to 180 days after treatment. Statistically significant improvement in outcome measures was demonstrated.

Black LL, et al. 2008. Effect of intraarticular injection of autologous adipose-derived mesenchymal stem cells on clinical signs of chronic osteoarthritis of the elbow joint in dogs. *Vet Ther.* 9(3): 192-200.

Black LL., et al. 2007. Effect of adipose-derived mesenchymal stem and regenerative cells on lameness in dogs with chronic osteoarthritis of the coxofemoral joints: a randomized, double-blinded, multicenter, controlled trial. *Vet Ther.* 8(4): 272-284.

STEM CELL CHRONICLE:
TO DIVIDE OR NOT TO DIVIDE



EXPERIMENTAL ANIMAL STUDIES SHOW POSITIVE RESULTS

1. Toghraie F., et al. 2012. Scaffold-free adipose derived stem cells (ASC's) improve experimentally induced osteoarthritis in rabbits. *Arch Iran Med.* 15(8): 495-499.
2. Guercio, A., et al. 2012. Production of canine mesenchymal stem cells from adipose tissue and their application in dog with chronic osteoarthritis of the humeroradial joints. *Cell Biol Int.* 36(2): 189-194.
3. Nicpon J., et al. 2013. Therapeutic effect of adipose derived mesenchymal stem cell injection in horses suffering from bone spavin. *Pol J Vet Sci.* 16(4): 753-754.
4. Zhang HN, et al. 2009. Uninduced adipose-derived stem cells repair the defect of full-thickness hyaline cartilage. *Chin J Traumatol.* 12(2): 92-97.

Except...

One horse study found, "Overall, the findings of this study were not significant enough to recommend the use of stem cells for the treatment of osteoarthritis"

Frisbie, DD., et al. 2009. Evaluation of adipose-derived stromal vascular fraction or bone marrow-derived mesenchymal stem cells for treatment of osteoarthritis. J Orthop Res. 27(12): 1675-1680.

This study concentrated the stem cells to 16.3 million cells, which is the equivalent of 1.63×10^7 ... which should be a good concentration when

compared to research mentioned above.

However, horses were also trotted on a high speed treadmill during this time, thus not allowing for the 'setting up' of a good scaffold of cartilage cells. Synovial fluid was collected weekly, thus re-establishing an inflammatory reaction each time, and the study was concluded at day 70, which was too short according to other studies that see success at the 6-month, 12-month and 2-year follow up marks. So, all in all, the study protocol did not set these horses up for success.

BAND WAGON



ARE YOU READY TO
JUMP ON THE STEM
CELL BAND WAGON?



SO, WHAT DO YOU THINK?

I think there is huge potential. I think we can be realistically optimistic about the outcomes too. I think we need to have a post-injection rehab plan (although it is likely quite similar if not the same to the PRP protocol that was created for you.) I think I'd be willing to try it if it were my or my dogs' joint(s). It will be exciting to see where regenerative medicine takes us in the future!

Until next time...

Cheers!



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Drop me a line! Send me your questions!



Four Leg Rehab Inc

PO Box 1581,
Cochrane, AB T4C 1B5
Canada

Laurie@Fourleg.com