



Regeneration of the Cranial Cruciate Ligament Using Regenerative Mesenchymal Stem Cells and Platelet Rich Plasma Therapy

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Abstract

Objectives: The purpose of this study was to evaluate the long-term histological revascularization and ligamentization of autogenous cranial cruciate ligament (CCL) replacement grafts in seven cats that had traumatic injury to the stifle, using stem cells and platelet rich plasma therapy. Twelve cats were treated with cranial cruciate ligament replacement graft without stem cell therapy were used as a control group to assess the efficacy of the stem cell therapy.

Methods: Radiographic images of the stifle joint were obtained prior to surgical repair of the ruptured CCLs.

Matured autogenous fascia lata grafts were harvested years later after they were used for CCL reconstruction.

A comparison was made between the histological results of the original fascia lata graft, and the matured fascia lata grafts in a group of nineteen cats that had traumatic injuries resulting from falling, or road traffic accidents.

Assessment of the efficacy of the stem cell therapy in this study was based on a comparison which was made between the treatment group who had stem cells therapy and the control group who did not have stem cells therapy, with respect to the clinical outcome and progress radiological findings, arthroscopic, histological and immunohistochemical examination findings.

Results: The result confirmed that the matured ligamentized fascia lata graft that was harvested from the seven cats that had stem cells and platelet rich plasma therapy had normal gross appearance and cellular structure indistinguishable from the native cruciate ligament. Return to normal function with no lameness and no pain or instability was achieved in the seven cats that had stem cell therapy within twelve weeks of surgery. However, the twelve cats in the control group who had no stem cell treatment continued to have mild/ moderate degree lameness and a mild /moderate degree of instability during the rest of their life.

Conclusions and Relevance: Stem cell therapy used in this study is an exciting new development, which helped to achieve good results in the treatment of CCL rupture in cats that had a history of significant trauma prior to the rupture of the CCL.

It was found that stem cell therapy helped to restore and regenerate the full mechanical and biological integrity of the ruptured CCL, which is the main stabilizing structure of the stifle.

Keywords

Cranial cruciate ligament rupture; Ligament regeneration; Stem cells; Revascularization; Innervation; Histology / immunohistochemistry

Introduction

Cranial cruciate ligament (CCL) disease in the dog is one of the most commonly diagnosed conditions that cause hind leg lameness in the dog. In comparison, relatively little has been written about cranial cruciate ligament rupture (CCLR) in the cat [1-4].

The prevalence of (CCLR) in the cat is likely to be under diagnosed since cats lead complex and busy lives. They explore, they hunt, they scavenge for food and they interact with other cats and are frequently seen crossing the roads unaided, this may be related to the difficulty in detecting lameness in cats, and spontaneous resolution without surgery occasionally seen in some cats [2,5-7].

Unlike in dogs, trauma is the most common cause of CCLR [8,9] and degeneration is unlikely to be an etiological factor for CCLR in cats [10]. Trauma can be caused as a result of sudden movement or torsion of the limb. This can occur especially in cats when jumping

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from heights or from awkward positions or collision with a vehicle or other objects. The injury that causes the cruciate injury in the cat also commonly results in tearing of one or both of menisci, collateral ligaments, posterior cruciate ligament and patellar ligament [8,9].

Occasionally the cat that has a ruptured anterior cruciate ligament will become sound (no longer showing signs of pain or lameness) even if surgery is not performed. However, if surgery is not performed within a few days to a week, arthritic changes will begin, that cannot be reversed or corrected, even with surgery this will result in lameness a few month later. If surgery is performed, it is possible that the cat will still develop arthritic changes in the stifle, but this will occur much more slowly and to a lesser degree than if surgery is not performed [8,9,11,12]. Furthermore, joint surgical stabilization has been suggested to reduce the incidence of meniscal tears in cats [5,6]. Surgical options involve various techniques such as ligament replacement using autogenous grafts [13] or synthetic prosthesis, stabilization with lateral capsular suture or fabellotibial suture [14-16].

Use of techniques such as ligament replacement or lateral capsular suture in cats were based on the fact that the injury was caused by trauma and the stifle was normal prior to injuries, this was confirmed from the clinical and radiographic examinations that were performed soon after the injury. The goal of the lateral suture technique relies on the successful stabilization of the joint until secondary periarticular fibrosis developed, as the suture stretches or break over time [17-19].

Preliminary results from a study by Minder [20] support the use TPLO in cats. Nineteen cats used in this study were mainly young cats (mean age of 5.5 years) and mean weight of (3.5 kg). Seven cats underwent surgical treatment using fascia lata graft, ligament regeneration stem cells and platelet rich plasma therapy for replacement of the damaged cranial cruciate ligaments and damaged collateral ligaments. Twelve cats that were treated with CCL replacement grafts without stem cell therapy were used as a control group to assess the efficacy of the stem cell therapy.

All nineteen cats were available for long-term follow-up (between 5 to 12 years). Degenerative cranial cruciate ligament disease does exist in older overweight cats [1-9]. While traumatic injuries affecting the CCL have been treated successfully in this study using fascia lata graft, stem cells and platelet rich plasma therapy, degenerative lesions of the cranial cruciate ligament may pose a challenge in regard to selecting treatment.

Materials and Methods

Animals

Nineteen cats that had a traumatic injury to the stifle and a complete rupture of the CCLS that were treated with autogenous fascia lata graft with or without stem cell therapy were enrolled to the study. Informed consent from the owners prior to enrolment into the study was obtained.

Only cats that had a definite traumatic incidence to the stifle were admitted to the study, all cats who had pathological changes in the stifle due to other causes were excluded from the study.

Prior to the surgical repair of the ruptured CCLs, the stifle joints were manipulated in flexion, extension, and rotation. The anterior drawer sign, and tibial compression test, were evaluated so that joint stability could be assessed.

Preoperative procedure

All radiological examinations, surgical procedures and arthroscopic examinations were performed under general anesthesia. Cats were premedicated with Acepromazine (0.01-0.02 mg/kg iv) (a). general anesthesia was induced with Alfaxalone (2-5 mg/kg iv) (b) and maintained with inhaled isoflurane (c) perioperative analgesia consisted of meloxicam (0.3 mg/kg sc) (d)

- a) Elanco animal health, Hampshire (uk).
- b) Jurox (uk)
- c) Merial animal health ltd, Harlow(uk).
- d) Boehringer Berkshire (uk).

Prior to the surgical repair of the ruptured CCLs radiographs were obtained of each stifle joint, Craniocaudal and lateral views of the stifles were obtained to exclude primary neoplasia of the distal femur or proximal tibia, to evaluate limb alignment and to determine tibial plateau angle (TPA) according to previously described method by Schnabl et al [21].

During the surgical repair of the ruptured CCLs, gross pathological and histological examinations were carried out on the original ruptured CCL. Biopsies of the original transplanted piece of fascia lata grafts were also obtained and histological and immunohistochemical examinations were carried out.

In seven cats, during CCL reconstruction, the fascia lata replacement grafts was impregnated with mesenchymal stem cells, which originated from adipose tissue and platelet rich plasma that was prepared from blood. Both were collected from the same cat and were sent to a specialized laboratory (Vet Laboratory / Vet Stem Therapy Laboratory) for processing. The adipose derived stem cells (ADSC)-platelet rich plasma (PRP) came back in vials in boxes at 4°C. 1 ml of ADSC-PRP was used to impregnate the fascia lata graft in each cat. Stem cells dose has ranged from 10 million cells / cat to 20 million cells / cat (A mean dose of 15 million Pluripotent adipose stem cells with tenogenic / ligamentogenic and chondrogenic differential potential lineage) were infused into the fascia lata cruciate ligament replacement graft. For stem cell differentiation into the desired cell lineage Gibco media was used. This was followed by pluripotent stem cell characterization to validate cells and to insure they are pluripotent and have been differentiated into the desired cell lineage for cellular characterization. PSC immunocytochemistry and live staining kits (OCT4, SSEA4) were used for cell identity confirmation authenticifer PCP amplification kits were used.

Concurrent disease states such as uraemia and full infectious disease testing and virology testing to include feline foamy virus and feline immunodeficiency virus were performed in each cat.

Arthroscopic examinations, a standard parapatellar portal arthroscopy was performed so a thorough knowledge of graft healing process is achieved, this was necessary for two reasons:

1. To assess the stage of graft healing (neovascularization and fibre pattern of the replacement graft) with a particular importance to accurately determine the amount of postoperative activity allowed, as subjecting the graft to excessive activity before vascularization and histological maturity will result in graft failure.
2. To decide if a second dose of stem cell therapy is required if healing was found to be slower and exhibiting a lesser degree of healing than expected. Postoperative analgesia consisted of meloxicam (0.05 mg /kg PO once daily) for 10 days.

Arthroscopy was performed once at three months postoperatively in all twelve cats who had no stem cell therapy and twice in all seven cats who had stem cell therapy, initially at 2 weeks, and then at 3 months postoperatively.

All 19 cats were subsequently euthanased between 5-12 years later for reasons unrelated to the study. Following euthanasia, at postmortem matured fascia lata autogenous grafts were harvested. In order to assess long-term histological outcomes of the CCL replacement grafts, the specimens were also examined with immunohistochemistry.

At postmortem, prior to harvesting of the matured fascia lata graft, the previously operated stifle joints were manipulated in flexion, extension, and rotation. The anterior drawer sign and

tibial compression test were evaluated so that joint stability could be assessed. Ventro-dorsal radiographs of the pelvis, and lateral radiographs of the stifles, were also obtained to assess progress in degenerative joint disease; this was based on assessing the degree in advancement in osteophyte formation as described by Trigari, Vaughan, Elkins et al, and Thomson et al [22-24].

At postmortem, the stifle joints were accessed through a minimal cranio-lateral approach. An incision was made in the joint capsule and mature fascia lata grafts were examined macroscopically. And the degree of osteophyte formation was recorded. This was followed by delicate removal of the CCL grafts. The sacrificed matured fascia lata grafts were sent to a specialist laboratory (FINN-Laboratory) for histological and immunohistochemical examinations.

At postmortem, in two cases, the un operated contra lateral stifles were used to compare the mature fascia lata replacement grafts with the normal ultrastructure of the CCL. The samples were fixed in 10% Formalin; later they were decalcified in EDTA and embedded in paraffin. The biopsy specimens of the matured fascia lata grafts were evaluated for vascularity, cellularity, fibre pattern, innervation and metaplasia. The histological examination and the gross pathological appearance results of the mature fascia lata grafts, were compared with the normal anterior cruciate ligament, and the original transplanted piece of fascia lata.

Results

Animals

Nineteen cats were used in this study. They were mainly young cats (mean age of 5.5 years) and with a mean weight of (3.5 kg). General clinical examination was unremarkable in all cats. Examinations for concurrent disease status, such as infectious disease and viral infection were negative. Orthopaedic examination that was performed on the stifles prior to surgical repair of the ruptured CCLs confirmed that apart from the ruptured CCL and severe cranial instability, which was confirmed in all cats by the anterior drawer sign and tibial compression tests and lateral instability in two cats. No other orthopaedic conditions were detected.

Radiographic Outcome

Radiographic examinations that were performed soon after the injury on the stifles confirmed that a part of the radiological signs that were related to the ligamentous injury such cranial displacement of the proximal tibia on the distal femur, which was visible in three cats without any stress being applied to the leg during the positioning and joint effusion which was seen as a reduction or obliteration of the fat pad and caudal displacement of the fascial planes, no other orthopaedic condition or neoplasia could be detected on the radiological examinations. Osteophytes which are signs of secondary osteoarthritis were not detected in any of the radiographs. Mean TPA in the 19 cats was 21.5 (range: 21-23). Radiographs that were taken years later, after surgical repair of the ruptured CCL using ADSC-PRP therapy and autogenous fascia lata grafts, showed slow and little advancement of the degenerative joint disease. In comparison the cats who did not have stem cells therapy, showed marked advancement of the degenerative joint disease.

Arthroscopic Examination Outcome

Arthroscopic findings at three months postoperatively in the twelve cats who had no stem cell therapy demonstrated poor neovascularization and development of the graft. In the seven cats who had regenerative mesenchymal stem cells, arthroscopy findings at 2 weeks postoperatively demonstrated that the transplanted fascia lata graft was covered with healing tissue and neovascularization was developing rapidly. Arthroscopy findings at 3 months postoperatively demonstrated fully intact CCL replacement graft, with marked neovascularization and normal fibre pattern.

Three out of the seven cats that had stem cell treatment required

a second dose of the stem cell therapy two weeks from the first dose as the arthroscopic examination showed that although healing was progressing nicely, it appeared to be slower than the other four cats. Arthroscopy at three months confirmed that healing was equal in all seven cats in this group of cats that had stem cell therapy.

Clinical Outcome

Clinical assessment of the stifles, prior to harvesting of the matured fascia lata in the group of 7 cats who had stem cell therapy, confirmed that all operated joints were stable in all directions, and anterior drawer sign, tibial compression tests could not be produced in any of the joints examined. However, in the control group of 12 cats that had no stem cell treatment, the operated stifles had mild / moderate cranial instability and the anterior drawer sign and tibial compression tests could still be produced.

Macroscopical and Histological Outcome

The gross appearance of the original CCL was found to be 'normal' but completely ruptured, with no apparent evidence of degenerative disease on histological examination, in all nineteen cats that had a history of a significant trauma prior to the rupture of the ACL.

Adipose derived feline mesenchymal stem cells in culture, showed the typical spindle - shaped fibroblast morphology (Figure 1). Histology of the original, transplanted piece of fascia lata, shows histological features consistent with a normal fascia lata, with its muscular attachment. Multiple tissue sections incorporate bundles of well-differentiated, mature striated muscle, with adjacent well-differentiated, well-vascularized, dense regular collagen bundles. The cellular component consisted of spindle fibroblasts and there was adjacent well-vascularized, unremarkable adipose tissue and multifocal nerve bundles (Figure 2). Vimentin (mesenchymal cells) of the fascia lata showed positive cytoplasmic staining of moderate numbers of spindle cells fibroblasts.

At postmortem, macroscopically the CCL replacement graft in the control group of the 12 cats that had no stem treatment were found to be ruptured, detached from the tibial and femoral interfaces, thinner than normal, with persistence of only a few fibres, weak, badly constructed and sclerotic. Microscopically, neovascularization was poor and located at the periphery of the grafts. No nerve tissue could be detected and collagenous fibres appeared disorganised. Degenerative lesions of the collagen fibres and chronic metaplasia changes were seen in-between one third and two thirds of the CCL replacement grafts.

In the seven cats who had stem cell therapy the CCL replacement grafts/matured ligamentized fascia lata were found to be intact throughout their entire length and fully incorporated at both the

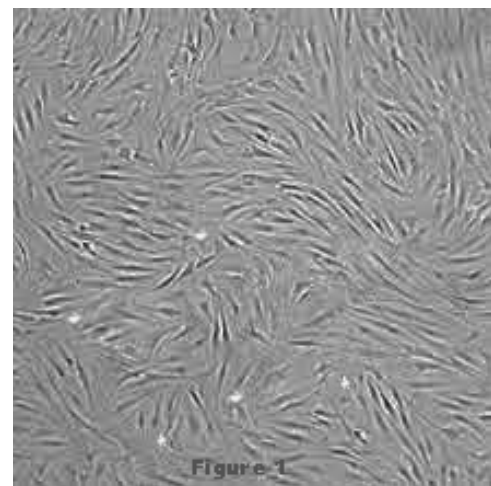


Figure 1: Feline mesenchymal stem cells in culture showing the typical spindle - shaped fibroblast morphology.

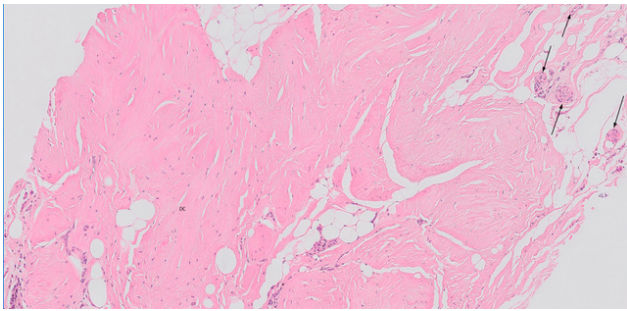


Figure 2: Histological sections of the original transplanted fascia lata, stained with H&E x 100, showing well-differentiated mature, striated, well vascularized (Black arrows), dense regular collagen poorly cellular, consisting of spindle fibroblasts. There is adjacent well vascularized adipose tissue and multifocal nerve bundles.

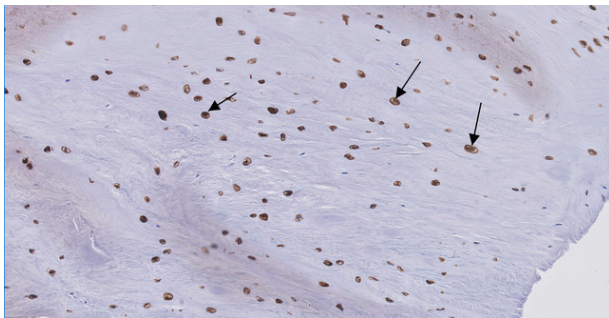


Figure 3: Multiple sections of the ligamentized fascia lata of the proximal and distal parts of the matured ACL graft, stained with vimentine IHC x 200, showing brown stained chondrocytes (arrows indicate some cells), incorporated in a dense collagen stroma; the blue background confirms that the ligamentized fascia lata had a structure of fibrocartilage.

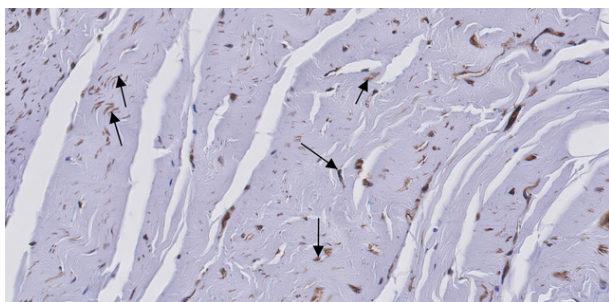


Figure 4: Multiple sections of the middle part of the ligamentized fascia lata, stained with vimentine IHC x 400, confirmed that this middle part contained more spindle shaped fibroblasts than the proximal and distal parts. Arrows indicate some of the spindle cells.

femoral and tibial interfaces. The mechanical properties and strength of the replacement grafts appeared to be able to restore normal function of the stifle joint. Macroscopically the ligamentized fascia lata appeared stout, sturdily constructed, conformed, and pliable.

During gross examination, the ligamentized autograft was found to be a fragment of tan to white, fibrous tissue, on average 5 x 1.5 x 0.5cm in size.

Histology of the Ligamentized Fascia Lata

The proximal and distal parts of the ligamentized fascia lata had a structure of chondral apophyseal entheses with a synovial lining.

They were solid, poorly cellular, and consisted of chondroblasts and fibroblasts. The fibroblasts were ovoid to round and resembled the cells of the articular cartilage; there were also some spindle fibroblasts. Abutting the dense, well vascularized collagen bundles, there were areas of fibrocartilage and a large number of chondrocytes were embedded within the fibro-cartilaginous ligamentized matrix (Figure 3).

Loose collagen bundles, with type II collagen fibers, were also found in these two fibrocartilagenous attachment areas, although they were more predominant in the proximal part than the distal part of the ligamentized fascia lata.

The middle part of the ligamentized fascia lata contained more spindle-shaped fibroblasts than the proximal and distal parts, signifying the spindle zone of the ligament (Figure 4). There were also multiple bundles of mature, well-vascularized, dense type I collagen fibers, with some intervening loose bundles of well-vascularized type II collagen fibres.

The collagen fibre density was higher in this middle part than the proximal and distal parts of the ligamentized fascia lata, and loose bundles of collagen type II fibres were mainly restricted to the fibrocartilagenous attachment site.

In some cases, a localized microgranuloma was incorporated within the dense fibrous connective tissue; this comprised a centre of slightly refractile amorphous material, surrounded by a fibrous connective tissue capsule, likely associated with a suture reaction, although no suture material remains (Figure 5).

Blood vessels were present in the normal fascia lata and the ligamentized fascia lata; this was confirmed with:

H&E [1], (Figure 6), Vimentin [2] (mesenchymal cells) staining (Figure 7), and, CD31 [3] (endothelial cells) staining (Figure 8).

There were an increased number of blood vessels, yet a reduced number of spindle cells in the ligamentized fascia lata, in comparison to the normal fascia lata.

Nerve tissue was present in the ligamentized fascia lata; this was confirmed with (1)H&E (Figure 9), (2) GFAP staining (Figure 10), and, (3)H7E, S-100 (Schwann cells) staining (Figure 11).

Longitudinal sections of the ligamentized fascia lata showed long, straight and parallel bundles of the dense collagen fibrils, in sequence with short segments where the fibril bundles suddenly change direction, forming a crimp pattern (Figure 12). Stretched tendon showed completely straightened collagen fibrils, in sequence with segments where the fibrils bend together forming knots, which correspond to 'fibrillar crimp' (Figure 13).

Fundamentally, the matured ligamentized fascia lata graft of the seven cats who had stem cells therapy appeared grossly, histologically, and immunohistochemically indistinguishable from the normal ACL,

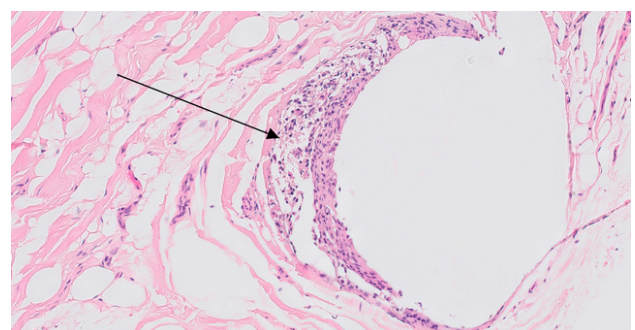


Figure 5: Section of the ligamentized fascia lata, stained with H&E x 200, showing microgranuloma (arrow), likely associated with a suture reaction, although no suture material remaining.

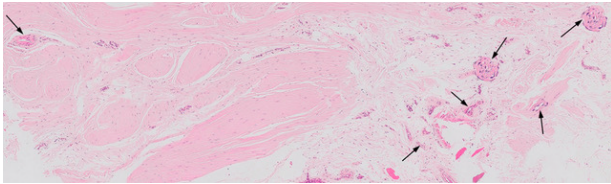


Figure 6: Sections of the mature ligamentized fascia lata showing vascular invasion of the graft, stained with Hematoxyline and eosine (x 100) showing multiple blood vessels of variable caliber (Black arrows).

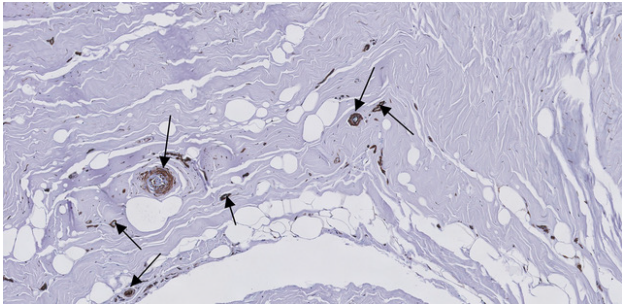


Figure 7: Sections of the mature ligamentized fascia lata showing vascular invasion of the graft, stained with vimentin IHC (x 100) showing brown stained blood vessels

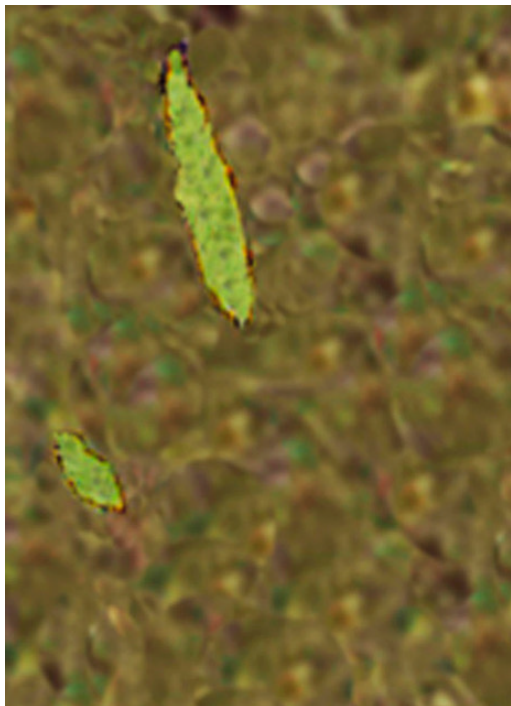


Figure 8: Sections of the mature ligamentized fascia lata showing vascular invasion of the graft, stained with CD31 (x 400) showing positively stained endothelial cells of the blood vessels.

with no signs of degeneration or host rejection even after it had been implanted for a period of up to 12 years. The space between the graft and the original bone was filled with a fibrovascular tissue at both the femoral and tibial interfaces.

Return to normal function with no lameness, and no pain or instability was achieved in the seven cats who had stem cell therapy

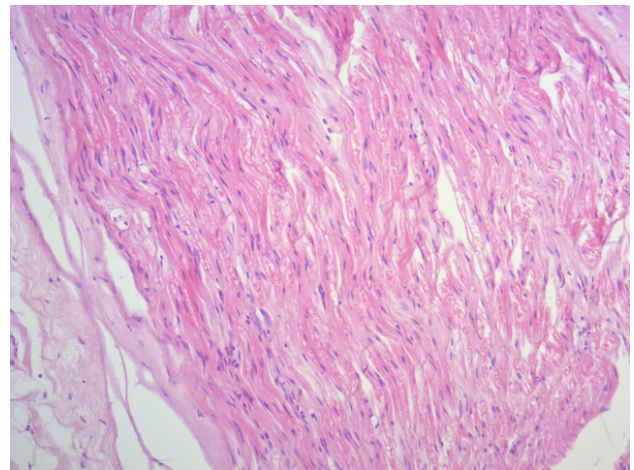


Figure 9: Sections of the mature ligamentized fascia lata confirming innervation of the graft stained with H&E (x 100) showing nerve tissue section

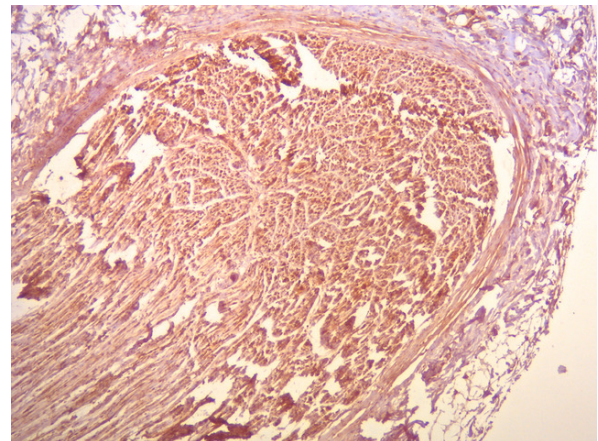


Figure 10: Sections of the mature ligamentized fascia lata confirming innervation of the graft stained with GFAP (x 400) Showing strong cytoplasmic staining

within twelve weeks of surgery. However, the twelve cats in the control group who had no stem cell treatment continued to have mild / moderate degree lameness and a mild / moderate degree of instability during the rest of their life. None of the cats used in this study had a ruptured CCL in the opposite stifle within their life span. No infection was seen in any of the cases.

Discussion

Assessment of the efficacy of the stem cell therapy in this study was based on a comparison which was made between the treatment group who had stem cells therapy and the control group who did not have stem cells therapy, with respect to the clinical outcome and progress radiological findings, arthroscopic, histological and immunohistochemical examinations findings.

Clinical Outcome and Progress Radiological Findings

The treatment of ruptured cranial cruciate ligament with fascia lata graft, and the use of regenerative mesenchymal stem cells and platelet rich plasma ADSC-PRP in seven cats produced excellent results with a long term satisfactory functional recovery. The limb was fully functional within six months of surgery, with a smooth recovery, and with a pain free life that lasted for years until they died from other causes. In contrast, poor clinical outcome was seen in the

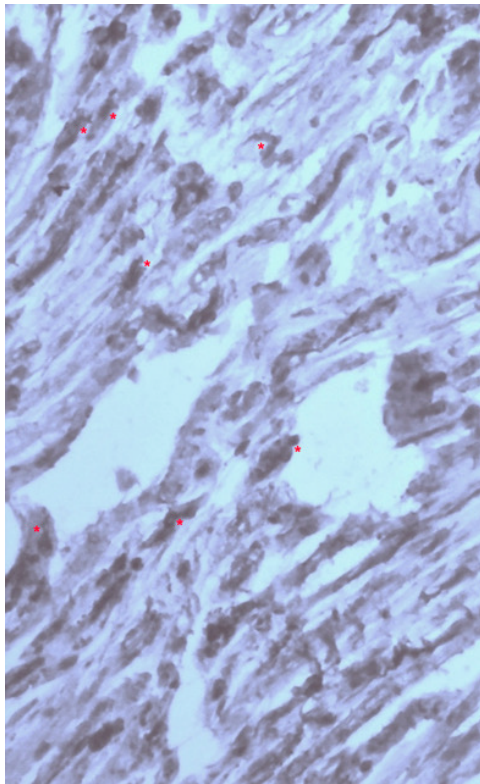


Figure 11: Sections of the mature ligamentized fascia lata confirming innervation of the graft stained with S- 100 (x 100) Showing positively stained glial Schwann cells (*)

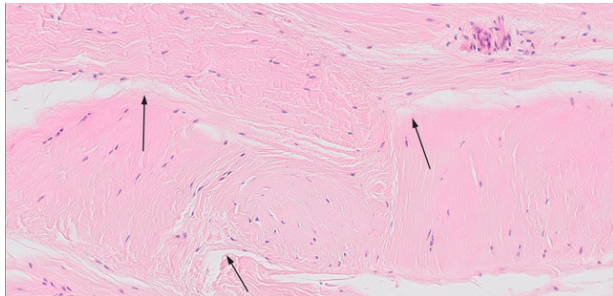


Figure 12: Histological section of longitudinally sectioned relaxed ligamentized fascia lata showing collagen fibril bundles arranged in a crimp pattern with sharp top angles evident in the crimp. Spindle cells are incorporated in the collagen fibrils H&E x 200.

twelve cats who did not have stem cells replacement therapy. They continue to have mild / moderate degree of lameness and a mild / moderate degree of instability during the rest of their life.

The stability achieved from the surgery in the group who had stem cell treatment restored the three essential functions of the normal anterior cruciate ligament by eliminating cranial subluxation of the tibia relative to the femur, internal medial rotation of the tibia with respect to the femur and hyperextension of the stifle; consequently neither anterior drawer movement nor tibial compression test could be produced. It is without doubt that any form of instability, either static or dynamic, will lead to progressive arthritis, pain and secondary changes such as meniscal injury.

In the seven cats that had stem cell therapy radiographs that were taken years later after surgical repair of the ruptured CCL showed little advancement of degenerative joint disease. In contrast to the

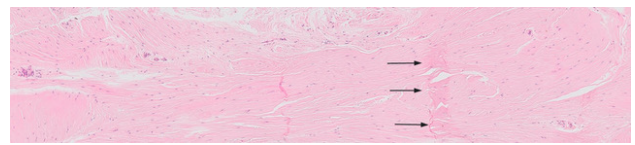


Figure 13: Histological section of stretched ligamentized fascia lata showing few segments of collagen fibrils, with partially stretched crimp corresponding to 'fibrillar crimps' H&E x 200.

twelve cats that did not have stem cell therapy, they all developed moderate to severe degenerative joint disease. This confirms that stifle stability which was achieved following the use of CCL autogenous graft replacement and ADSC-PRP has definitely slowed down the arthritic changes in the operated joints.

Mean TPA in the nineteen cats was 21.4 (range 21-23). Which is similar to that previously reported by Schnabl et al. [21] in cats that do not have any evidence of cranial cruciate ligament injury. This may be related to the absence of any abnormal conformational changes in the proximal epiphyses of the tibia and the stifles were essentially normal prior to the traumatic rupture of the CCL, it does also make fascia lata graft with stem cell therapy an acceptable choice of stabilization. Larger series are required for further evaluation.

Arthroscopic Examination Findings

Arthroscopic examination has demonstrated that the time required for the development of neovascularization of the CCL ligament replacement graft in the seven cats who had regenerative ADSC-PRP therapy was 2 weeks, and the time required for complete

ligamentization of the graft was 3 months. In contrast, arthroscopic examination in the twelve cats who had no stem cell therapy demonstrated poor neovascularization and development of the graft.

Previous report by Giordano et al. [25] showed that ligamentization without stem cell treatment did not take place until at least 24 months postoperatively. It may be that the use of the platelet-rich plasma with the plasma cells has a role to play in achieving this result. A recent study reported that platelet rich plasma provides growth factors to enhance and promote stem cells engraftment, providing a synergistic effect to the stem cells, to proliferate and stimulate its differentiation into fibroblast [26]. These findings promote the use of PRP in combination with stem cells to aid ligament healing and maturation and accelerate healing. Arthroscopic examination in this study was important to accurately determine the amount of postoperative activity allowed, as subjecting the graft to excessive activity during the initial stage of necrosis or before vascularization and histological maturity will result in graft failure [25,27,28] and to decide if a second dose of stem cell therapy was required, if healing was found to be slower and exhibiting a lesser degree of healing than expected in the group of cats who had stem cells treatment.

Histological and Immunohistochemical Findings

Histological and immunohistochemical examination findings in the seven cats who had regenerative mesenchymal stem cell treatment were consistent with those of native and intact cruciate ligaments in terms of vascularity, cellularity, fibroblast arrangement, and collagen fibre organisation, i.e. in a collagen crimp and strict parallel alignment pattern. These findings were similar to those of Giordano, NgG Y et al, Jansen and Scheffier [25,29,30]. Ligamentization in the seven cats who had regenerative mesenchymal stem cell treatment was due to the significantly more advanced neovascularization of the fascia lata graft. This improved growth and migration of the fibroblasts to the ligamentization site. Nearby fibroblasts proliferate and migrate to the ligamentization site and produce large amounts of collagenous matrix and collagen fibrils. Biomechanical change from

the spindle-shaped fibroblasts to the round shaped chondrocytes occurred at the proximal and distal end of the ligamentized graft. This was induced, at least in part, by the change in cell shape and attachment to the surrounding matrix produced by the preexisting chondroblasts [31,32]. This histological change allows a graduated increase in stiffness from the ligamentous tissue to the rigid bone and may prevent stress concentration at the attachments site.

It appeared that the histological composition of the transplanted, normal fascia lata, which consist of dense regular collagen, had undergone metaplastic change. The immunohistochemistry showed that the spindle mesenchymal cells of the transplanted fascia lata had undergone a morphological change, had transformed, and had increased in number to chondrocytes to incorporate fibrocartilage, which would normally be present in the cruciate ligament. There was also an increased number of blood vessels within the ligamentized fascia lata, in comparison to the transplanted fascia lata, which supply the essential and adequate demand of vascularization for the metaplastic change. In addition, there were more spindle cells in the normal fascia lata than the ligamentized fascia lata; spindle cells are responsible for the synthesis of collagen and extracellular matrix; therefore, they are essential for the transformation and creation of a strong ligament to replace the ruptured one.

In contrast, poor histological outcome was seen in CCL replacement grafts of the twelve cats who had no stem cell replacement therapy. They were found to be ruptured with poor neovascularization, absent innervation and disorganised collagenous fibres.

Large number of stem cells replacement therapy was used in the seven cats in the treatment group. As a percentage of the stem cells only survive for a very short time after they have been infused, the remaining cells seem to proliferate, differentiate and renew themselves for a long period of time. Scientists are trying to understand what factors regulate stem cells early degeneration. A number of studies showed that the number of stem cells used were positively correlated to the level of success of stem cell therapy [33-35].

Multipotent stem cells with Tenogenic / ligamentogenic and chondrogenic lineage have been used in this study. The use of multipotent stem cells which can specialize into specific lineage when cultured for a specific clinical condition is of prime importance in the outcome of stem cell therapy [36-43]. Adipose subcutaneous stem cells were used in this study as they are easily accessible and can be isolated without clinical risk [41].

Although stem cells have initially been administered intravenously [44]. The intralesional route was used in this study, the best possible route of stem cells administration will be based on whether the cells need to act locally or systemically, to achieve the clinical and therapeutic objective [35]. Adverse events of illness, feline foamy virus, feline immune deficiency virus which were described by Webb et al. [45] and Quimby and Borjesson, [35] could not be found in any of the cats admitted to the study.

Our results provide additional evidence to support the use of stem cell treatment in cruciate ligament disease, found in previously reported case studies in humans [46-49]. They confirmed that stem cell therapy helps to restore and regenerate the full mechanical and biological integrity of the anterior cruciate ligament.

Explanation of how stem cells therapy restores the mechanical strength of the native cruciate ligament is quite fascinating. It was found that stem cell therapy is a mechanical stimulus that is mediated via stem cell differentiation into fibroblast-like cells, as seen by the upregulation of ligament markers: Tenascin-c, collagen types 1 and 111, and the formation of collagen fibres, as well as the production of extracellular matrix (ECM) [50,51]. The main limitations of our study are related to the use of a small group of cats. Further studies are needed to determine if our results are reproducible in a larger group of animals, to include a randomised, blinded and controlled trial.

Conclusion

The overwhelming success achieved in the cases described in this

study, by using ADSC-PRP, the histological change of the autograft into an identical structure to the normal CCL, and, that it remained successfully incorporated for up to twelve years, supports the use of ADSC-PRP with autograft ligament replacement for the treatment of cruciate ligament rupture in cats that had a history of significant trauma prior to the rupture of the CCL.

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