

AdiCell™ and adipose-derived cell therapy

1. The AdiCell™ procedure

First fat is collected from the patient. The fat, also called adipose tissue is processed to separate the cells from the connective tissue. The process uses an enzyme preparation that breaks down collagen, the fibre that binds the fat tissue together.

The cells are then washed by centrifugation to remove the enzyme. The cells are then ready for injection into the damaged joint. The entire process takes less than 2 hours.

2. Safety

Whilst AdiCell™ is a new technology; there are a number of procedures that have been in use for many years that are quite similar to the AdiCell™ procedure. Bone marrow transplants (Hematopoietic Stem Cell Transplantation) have been in common use on humans since the 1950s. Whilst this is not exactly the same as the AdiCell™ procedure, it does inform us on the areas of safety and efficacy.

The other area, which is quite close to the AdiCell™ procedure, is the practice of fat-transfer in cosmetic and reconstructive surgery. This is typically a liposuction procedure for fat removal, followed by minimal processing, often just washing and centrifugation. The tissue is then transplanted back into the donor. The cosmetic and reconstructive surgery industry is one of the fastest growing medical disciplines worldwide and the safety and efficacy of these procedures is not in question. This is because the tissue for transplantation is **autologous** and very minimally processed.

Autologous means that the tissue is transplanted back into the same person. This is known as an autologous transplant. When tissue from a different person is used it is called an allogeneic transplant.

AdiCell™ is an autologous procedure and the tissue is minimally processed.

There is such a large amount of clinical and basic research data available on autologous transplants of minimally processed tissue that Regeneus and its partners are in a unique position: the AdiCell™ approach is in the safest category of cellular therapies.

3. Veterinary Partnerships

Through partnerships with respected veterinarian practices, pet owners can access AdiCell™ through a network of well-equipped, world class veterinary practices that offer exceptional client and patient care. Selected and accredited vet clinics offer AdiCell™ to dogs suffering from osteoarthritis.

Regeneus only partners with the highest quality veterinary practices and implements a quality assurance program within the veterinary practice that ensures the quality of every AdiCell™ procedure. Regeneus monitors the quality assurance program using independent auditors.

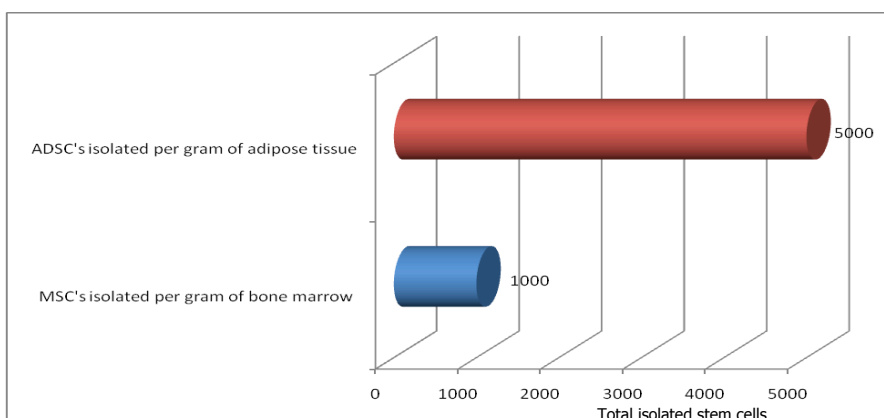
4. Adipose derived stem cells

Adipose tissue is filled with cells called adipocytes. Adipocytes are cells that store fat and as an individual becomes more obese the adipocytes swell as they hold more fat. It has recently been recognized that these adipocytes play an important cell-signalling role and secrete a range of substances that effect many parts of the body.

Running throughout the adipose tissue is a network of capillaries that deliver nutrients and oxygen to the adipocytes and carry away the secretions. Hanging off the capillaries, like bunches of grapes on a vine, are large numbers of cells that are not adipocytes. These cells include stem cells.

There are many publications in the scientific literature that demonstrate without doubt that mesenchymal stem cells exist in fat in high numbers in fat. In fact, stem cells exist in fat in far higher numbers than in bone marrow.

Figure 1: Relative abundance of stem cells in adipose tissue and bone marrow aspirate



Strem BM, et al., *Kieo J Med* 2005 54: 132-141

Stem cells are not the only cells that are attached to the capillaries that run through adipose tissue. There are endothelial progenitor cells known to be involved in angiogenesis, monocytes and macrophages that secrete anti-inflammatory cytokines, pericytes, mast cells, preadipocytes, fibroblasts, and smooth muscle cells. The AdiCell™ procedure utilises this entire mixture of cells along with the stem cells.

It is widely accepted that for a stem cell therapy to be effective there must be reasonably high numbers of stem cells used for the treatment. It is normal practice that to obtain sufficient cell numbers, cells are cultured for several weeks. A big breakthrough in the development of stem cell treatments occurred when it was discovered that adipose tissue contained very large numbers of stem cells. This discovery meant that sufficient cells for a treatment could be directly harvested from adipose tissue. Up until this point stem cells had to be grown and amplified in culture to produce sufficient numbers for an effective treatment.

When cells are cultured they change. These changes are not well understood and carry risks such as cancer and immune rejection.

By using cells directly harvested from adipose tissue there is no need to culture the cells because sufficient numbers of cells can be easily collected. This means that there are less safety issues and that the treatment is rapid and does not require specialised equipment for culturing cells.

The relatively non-invasive techniques required to recover adipose derived stem cells (ADSC's) combined with the higher stem cell numbers recovered makes adipose tissue a safe and effective option.

Other types of stem cell treatments carry significant risks. For example, a recent publication described how a patient treated with allogeneic neural stem cells developed tumors that were derived from multiple donors, including both male and female donors.

Amariglio N, Hirshberg A, Scheithauer BW, Cohen Y, Loewenthal R, et al. (2009) PLoS Med 6(2): e1000029. doi:10.1371/journal.

5. **AdiCell™ and the treatment of Osteoarthritis**

In treating osteoarthritis there are two distinct areas, which need to be addressed. The first is pain and inflammation. The second is cartilage regrowth. In the literature these two areas are usually investigated separately, however, the strength of the AdiCell™ procedure is its ability to address both short-term pain and inflammation issues and maintain the improvement over long periods of time.

Pain and inflammation

In the first area of pain and inflammation, it is now known that the secretions of mesenchymal stem cells have positive effects on inflammation and cellular stress. This anti-inflammatory effect is predominantly generated through four primary mechanisms:

1. Inhibition of the function of memory T-cells
2. Secretion of cytokines including IL-10
3. Increased regulatory T-Cell population
4. Prevention of antigen presenting cell development

In general, the very broad literature on MSCs and secretions describes positive effects not only on inflammation, but also angiogenesis, wound healing and decreased cell death after ischemia.

Treatment of animals suffering osteoarthritis with the AdiCell™ treatment have shown significant reductions in pain over a period of 6 months. The reduction in pain was larger than that reported for a commonly used non-steroidal anti-inflammatory drug carprofen (Figure 3). Furthermore, these results have been replicated in a double blind study carried out by Black *et al.*, (2007) Veterinary therapeutics. Vol 8, No.4.

Figure 2. Improvement in pain after AdiCell™ treatment

Treatment	Improvement in pain
AdiCell™ at KVH	1.7
US double blind study using adipose derived cells	1.57
Carprofen – double blind study ¹	1

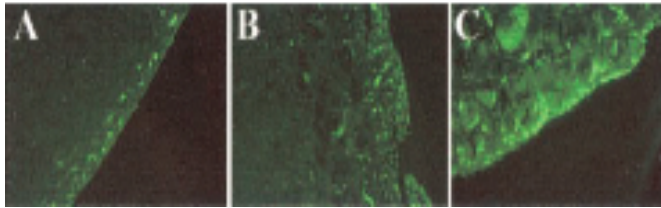
¹Osteoarthritis and cartilage 9 (suppl B):S21-22

The longevity of the observed reduction in pain can be contributed to the ability of the transplanted cells to become localized to the affected area, where there is a continuous production of these anti-inflammatory effects.

In a study in goats, MSC's were labelled with a fluorescent tag and injected into damaged joints. Figure 4

shows the localization of the fluorescent cells in the joint 12 weeks after treatment.

Figure 3. Retention of Cells in the joint



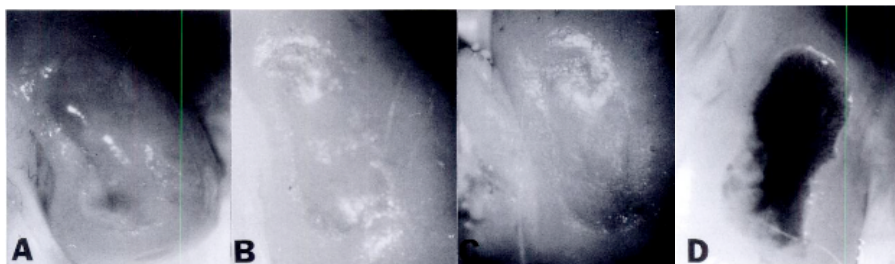
Cells detected in the meniscus (A), synovial capsule (B), and the periosteum on the medial aspect of the medial condyle (C).

Murphy *et al.*, 2003. *Arthritis and Rheumatism*, 48: 3464-3474

Tissue regrowth

There is substantial literature demonstrating cartilage (and other tissue) regrowth in animal models, one of the most quoted is Wakitani *et al.* *J Bone Joint Surg Am.* 1994;76:579-592. This study involved rabbits which had holes drilled through the articular cartilage into the sub-chondral bone. The effects of implantation of mesenchymal stems cells on the infected joint were examined at various time points (Figure 5).

Figure 4. Macroscopic appearance of defects at different intervals of healing following implantation of mesenchymal stem cells



6x3 defects created in rabbit knee cartilage

A = 2 weeks post treatment

B = 4 weeks post treatment

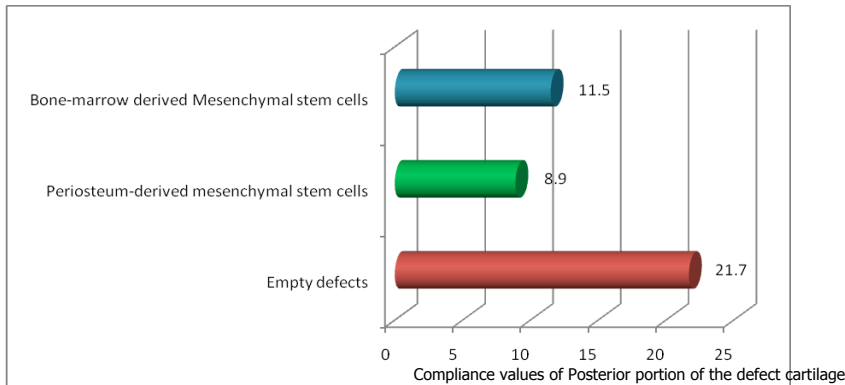
C = 24 weeks post treatment

D = No cells

Wakitani *et al.* *J Bone Joint Surg Am.* 1994;76:579-592

Further compelling data from this study relates to the mechanical properties of the regenerated cartilage (Figure 6). Small numbers (usually single digits) imply normal, low compliance (high stiffness); while large numbers (approximately twenty) indicate that the tissue is softer and more compliant than normal articular cartilage in the rabbit.

Figure 5. Compliance values of the tissue 24 weeks after Treatment



Mechanical testing of cartilage compliance

The lower the value the closer the compliance to normal tissue

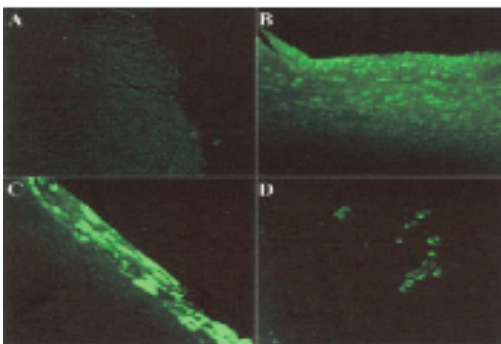
BM and Periosteum-derived cells produced significant improvement in compliance

Wakitani et al. J Bone Joint Surg Am. 1994;76:579-592

The cell implants produced tissue; which approached that of normal cartilage. Certainly by this measure the cell free "no-treatment" groups produced very soft tissue, which was over twenty on this measurement scale.

Further evidence supporting tissue regrowth occurring in MSC treatment, has been generated in studies on osteoarthritis induced in goats by complete excision of the medial meniscus and resection of the anterior cruciate ligament. GFP tagged mesenchymal stem cells injected into joint have been detected in the regenerated meniscal tissue (Figure 7).

Figure 6. Microscopic analysis of Regenerate mensial tissue



GFP positive cells were detected primarily at the surface (B and C) and also in the center (D) of neomeniscal tissue 6 weeks after injection of GFP mesenchymal stem cells. Negative control (A).

Murphy *et al.*, 2003. Arthritis and Rheumatism, 48: 3464-3474

Two papers published in 2008 by Centeno et al, discuss the regeneration of cartilage volume in a human patient. The conclusion reached is: The described process of autologous mesenchymal stem cell culture and percutaneous injection into a knee with symptomatic and radiographic degenerative joint disease resulted in significant cartilage growth, decreased pain and increased joint mobility in this patient. This has significant future implications for minimally invasive treatment of osteoarthritis and meniscal injury.

6. **Summary**

AdiCell™ is an advanced technology that uses the dog's own natural regeneration system to heal painful arthritic joints. Unlike major surgery, it is a non-invasive, one-day procedure and involves implanting cells into affected joints. AdiCell™ has demonstrated remarkable results in the dogs treated so far, with the increase in mobility and the concurrent reduction in pain and inflammation mirroring results obtained in the scientific literature.