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List of abbreviations

ACD-A	Acid Citrate Dextrose A.
ACI	Autologous Chondrocyte Implantation
AD-MSCs	Adipose Tissue Derived Mesenchymal Stem Cells.
BM-MSCs	Bone Marrow Derived Mesenchymal Stem Cells.
CPM	Continuous Passive Motion.
ECM	Extra Cellular Matrix.
FGF	Fibroblast Growth Factor.
GFP	Green Fluorescent Protein.
IACUC	Institutional Animal Care And Use Committee.
JSDs	Joint Surface Defects.
LLLT	Low Level Laser Therapy.
MSCs	Mesenchymal Stem Cells.
NGF	Nerve Growth Factor.
OA	Osteoarthritis.
PBS	Phosphate Buffer Saline.
PDGF	Platelet-Derived Growth Factor.
PRP	Platelet Rich Plasma.
SDF-1	Stromal Cell-Derived Factor-1 Alpha.
SVF	Stromal Vascular Fraction.
TGF-beta	Transforming Growth Factor beta.
VEGF	Vascular Endothelial Growth Factor.

Introduction

Hyaline articular cartilage plays a crucial role in mammalian joint function. Based on its distinct histo-anatomical structure it allows nearly frictionless movement between articulating bones while reducing peak stress forces during joint load at the same time **(Hunter, 1742).**

Hyaline articular cartilage is avascular with no neuronal or lymphatic supply (**Buckwalter et al., 2005**)**.** Due to the lack of vascularization and consequent lack of access to a pool of potential reparative cells and growth factors, cartilage response to injury does not follow the typical necrosis, inflammation, repair and scar tissue remodeling cascade of events. As a result, its endogenous healing potentials are limited, and any structural damage, even a penetration of the subchondral bone plate occurs, is usually irretrievable and is repaired with fibrous or fibrocartilaginous tissue (**Buckwalter & Brown, 2004**)**.** True restoration with repair tissue mimicking hyaline cartilage was never observed in humans or in other mammalian species (**Buckwalter et al., 2005**)**.**

Joint injury is the most common cause of chronic pain in dogs, with more than 20%, or 10 to 12 million dogs worldwide afflicted **(Denny and Butterworth, 2000).** This is characterized by synovitis and degeneration of the articular cartilage with loss of matrix and can result in complete loss of the cartilage surface **(Black et al., 2008)**.

The end stage of joint injury is Osteoarthritis (OA). In OA, there exists an overproduction of destructive and pro-inflammatory mediators relative to the inhibitors, resulting in a balance in favor of catabolism rather than anabolism, which in turn leads to the progressive destruction of articular cartilage (**Buckwalter et al., 2005**).

Advances in understanding of the biology of adult stem cells have attracted the attention of the biomedical research community, including those studying OA. The interest given to adult stem cells is due to the facts that they are immunologically compatible, can be harvested from a variety of sources, including bone marrow and adipose tissue **(Luyten, 2004)**, and have no ethical issues related to their use.

Adult stem cells, such as Mesenchymal stem cells (MSCs) derived from bone marrow (BM-MSCs) and adipose tissue (AD-MSCs) are the most highly characterized cells and are considered comparable. Both have demonstrated broad multipotency with differentiation capabilities into a number of cell lineages, including adipo-, osteo-, and chondro-cytic lineages **(Parker and Katz, 2006)**.

However, the easiness and repeatable access to subcutaneous adipose tissue, the effortless isolation procedure, and the greater numbers of fresh MSCs derived from equivalent amounts of fat versus bone marrow (approximately 500-fold) provide distinct advantages in using AD-MSCs over BM-MSCs **(Schäffler and Büchler, 2007).** In addition, the isolated AD-MSCs can be easily cryopreserved **(Fraser et al., 2006)** yet maintains high growth rate and differentiation capabilities after resuscitation **(Tsuji et al., 2014)**.

Clinically, Stromal Vascular Fraction (SVF) derived from adipose tissue have the advantage over their bone marrow-derived counterparts, because of their abundance in numbers eliminating the need for culturing over days to obtain a therapeutically viable number and the ease of the harvest procedure itself being less painful than the harvest of bone marrow **(Strem et al., 2005).** This, in theory, means that an autologous transplant of adipose-derived stromal cells will not only work in much the same way as the successes shown using marrow-derived mesenchymal stem cell transplant, but also be of minimal risk to the patient.

The AD-MSCs regenerative potentials can be promoted through adding growth factors. Platelet Rich Plasma (PRP) is the blood plasma enriched by platelets. It contains many growth factors and has been clinically and successfully used to improve hard and soft tissue healing **(Andia and Abate, 2013)**. Recently, the PRP was proven to enhance the proliferation of AD-MSCs (**Van Pham et al., 2013**)**.**

In order to maximize the cellular proliferation efficiency of AD-MSCs in PRP vehicle, low level laser irradiation was used. Laser irradiation at different intensities has been recognized to inhibit and/or stimulate cellular processes. Recent findings suggest that at the cellular level, laser energy of a particular wavelength can initiate signaling cascades, such as those that promote cellular proliferation **(Mvula et al., 2010).**

The aim of this work is to evaluate the intraarticular injection of photoactivated adipose derived stem cells added in a platelet rich plasma vehicle as a single step surgical procedure for the treatment of experimentally induced partial thickness chondral defect in dogs.

Review of the literature

I. Chondral defects:

I.1. Prevalence:

Joint surface defects (JSDs) are focal lesions of the articular cartilage. They are very common, being reported in about 20% of all human arthroscopic procedures **(Buckwalter and Brown, 2004)** and 32% of equine and canine arthroscopies **(Dell'accio and Vincent, 2010).** They are clinically important as they can be symptomatic and disabling, with pain and/or locking of the joint, and can predispose to further cartilage loss and development of OA (**Buckwalter and Brown, 2004**).

Moreover, up to 43% of healthy human patients without a history of OA have knee chondral lesions as evaluated by Magnetic Resonance Imaging (MRI) **(Dell'accio and Vincent, 2010b).**

These data point to the fact that chondral or osteochondral defects are more common than previously thought, are not necessarily of traumatic origin, and need not be symptomatic.

I.2. Types & causes:

- **Traumatic Focal Articular Cartilage Defects (Traumatic arthropathy, chondral defects)**

An articular cartilage injury occurs when there is trauma to the joint surface. This most often occurs when the bones are forced to slide across one another or impact each other with marked force. This can occur following a twisting mechanism, as happens at the time of an anterior cruciate ligament rupture **(Ye et al., 2013).** Articular cartilage defects of the patella frequently occur following a fall with direct impact to the front of the knee or a dislocation of the patella (**Buckwalter and Brown, 2004**) **.**

- **Chondromalacia (localized wear and tear)**

Softening or fissuring of the articular cartilage usually a consequence of cumulative injury or degenerative joint disease. Very common in old human patients and animals **(Anderson et al., 2011).**

- **Osteochondral fracture (joint cartilage and bone involvement)**

A hard blow to the knee may cause a piece of articular cartilage to break off and pull with it a piece of its underlying bone. The broken off fragment may end up free floating in the joint "loose body". A loose body may rub against the tissues within the knee, causing pain and locking. If large enough, the loose fragment of cartilage/bone may be re-attached into its original position, as to a jigsaw puzzle, and secured with special anchors. If the fragment is too small it is discarded and the remaining crater managed according to its size **(Panseri et al., 2012).**

- **Degenerative Joint Disease: (osteoarthritis, arthrosis)**

When the knee surface has most of its surface damaged or a large portion of a major weight bearing the knee is called arthritic. The articular cartilage wear is extensive and severe and the knee function deteriorates. In some instances the cartilage degeneration is due to abnormal alignment causing more wear on one side than another of the knee **(Rettenmaier et al., 1995).**

I.3- Pathology and healing process of chondral defects:

As the articular surface is avascular, the normal cycle of tissue repair cannot proceed. Without a blood supply, no hematoma is formed, fibrin is not produced, and the inflammatory phase doesn't occur (**Buckwalter, 1998**). In addition, without a fibrin network, the repair phase is very limited. The small amount of repair is generated solely by the remaining viable chondrocytes, as no supply of undifferentiated cells is available. These chondrocytes proliferate, but their response is incomplete and short-lived.

Chondrocytes, the only cells of articular cartilage, maintain homeostatic synthesis and degradation of the extracellular matrix via the secretion of macromolecular components (collagen, glycosaminoglycan, and hyaluronic acid) and modulation of the extracellular matrix turnover. Chondrocyte secretion of lytic and tissue-damaging mediators (cytokines, free radicals, proteases, prostaglandins) are controlled by a balance of anabolic and reparative substances (growth factors, inhibitors of catabolic cytokines) and inhibitors of degradative enzymes **(Denny and Butterworth, 2000).**

Small superficial lesions typically remain stable over time and do not progress to degenerative arthritis (**Buckwalter et al., 2005**). Larger lesions with articular flaps and loose articular debris, however, cause joint effusion with mechanical symptoms and have the potential to progress to significant degenerative arthritis. Blunt trauma to articular cartilage is a particularly insidious injury. Initially, the gross appearance of the articular surface may be normal; however, the microscopic examination will reveal disorganized collagen, increased water concentration, and decreased proteoglycan concentration (**Buckwalter, 1998**). This type of blunt articular injury can later progress and degenerate.

The development of osteoarthrosis goes through five stages:
-Breakdown of articular surface.
-Synovial irritation.
-Remodelling.
-Eburnation of bone and cyst formation.
-Disorganization.

Breakdown of articular surface

Degenerative osteoarthritis begins with failure of the articular surface. The normal smooth surface of articular cartilage is breached, the arcades of collagen fibers break and the surface becomes rough, like a shaggy carpet. Friction against the rough surface generates particles of articular cartilage that are shed into the joint and absorbed by the synovium, where they cause an inflammatory response which the patient feels as stiffness or aching in the joint after exercise rather than at the time of use **(Desrochers et al., 2010).**

Synovial irritation

The irritation of the synovium is probably due to the release of intracellular enzymes, including lysozymes, which produce hyperaemia and a cellular response in the synovial layers. The synovium can also produce degradative enzymes and mediators such as interleukin-1, which may influence chondrocyte activity. Other potential causes of damage include free radicals and the deposition of immune complexes **(Dell'accio and Vincent, 2010).**

Remodelling

Limited cartilage repair can occur. Superficial lesions of articular cartilage show little healing but deep lesions that penetrate cortical bone allow the influx of marrow cells and the formation of fibrocartilage. Hyaline cartilage, however, is a once-in-a-lifetime tissue and does not regenerate **(Ye et al., 2013).**

The subchondral bone is also abnormally active, with an increase in both the density of the tissue and the number of cells. At the margin of the joint, new bone forms as osteophytes covered by fibrocartilage, perhaps induced by wear particles swept to the edge of the joint by joint movement. Therefore, the osteophytes restrict joint movement (**Andia et al., 2012**)**.**

A line of dense, hard, resilient bone forms just below the cartilage and the joint 'remodels' so that there is a change in shape and congruity. This alters the pattern of weight-bearing, which in turn means that the load is taken by different areas of articular cartilage **(Breinan et al., 1997).**

Eburnation of bone and cyst formation

If the joint is rested, the wear particles are gradually absorbed. Fibrous tissue may form in the defect on the joint surface but as time goes by this repair process gradually fails and the articular surface is eroded to expose subchondral bone, which subsequently becomes polished and eburnated **(Denny and Butterworth, 2000; Gotterbarm et al., 2008).**

Raw bone rubbing against raw bone is painful. The eburnated bone is not as slippery as healthy articular cartilage, friction across the joint is increased and weight transmission across the joint becomes uneven. This change overloads some parts of the joint surface and microfractures occur in the trabeculae of the cancellous bone **(Gotterbarm et al., 2008).**

The microfractures heal with callus, which increases the rigidity of the bone so that the bone gradually becomes denser, more sclerotic and less resilient. This, in turn, causes more microfractures and the normal architecture of the bone is lost (**Buckwalter and Brown, 2004**).

At this stage, synovial fluid enters the cancellous bone under pressure through cracks in the articular surface, producing cavities that are seen radiologically as ‘cysts’. These cysts fill with fibrous tissue and become lined with a thin shell of cortical bone.