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ARTICULAR CARTILAGE REPAIR IN ANIMALS AND ITS PATHOPHYSIOLOGY - A REVIEW

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ABSTRACT

Cartilaginous tissue which somehow gets injured heals slowly or incompletely as it has very limited capacity by its own to heal. Immense researches on cartilage regeneration have been performed by many researchers in the past on humans as well as on animals. Attempts have been made to replace the damaged cartilage with new functional tissues but almost all such efforts done in the past have remain in vain due to the lack of understanding and knowhow of reparative physiology of the cartilage at molecular level. Lately, different modules including scaffolding and cell therapy approaches have been tried as a novel strategy in the treatment of cartilage damage or diseases. Many progenitor cells have been tried in the past such as mesenchymal, embryonic, adult, bone marrow stromal and adipose derived stem cells in the repair of many tissues including the articular cartilages besides their efficacies and limitations. This review focuses on the physiological insight of repair biology of cartilage in racing and sporting animals in the light of newer modules of treatment available.

Keywords: Cartilaginous tissue; Differentiation; Trans-differentiation, Mesenchymal stem cells, Embryonic stem cells, Bone marrow stroma stem cells, Adult stem cells; Adipose tissue derived stem cells; Scaffolds

INTRODUCTION

Lameness is the most important cause of wastage among draft and sports animals (Rosedale *et al.*, 1985) and in fact the fore fetlock has the largest number of site–specific traumatic and degenerative lesions of all joints of the appendicular skeleton (Pool, 1996). Furthermore, developmental defects such as osteochondrosis in racing animals can lead to osteochondritis dissecans. As a result of the same cartilage flaps, fissures and poorly organized subchondral bone produce disruption of joint surfaces in such animals. Articular cartilages have remarkable function especially during motion. Articular cartilages are having unit

cells as chondrocytes which are residing in the cartilaginous special matrix. So far, articular cartilage has not been duplicated as the native structure (Douglas *et al*., 2001).

Articular cartilage is prone to factors such as joint trauma, chemical and mechanical exposures that can lead to its eventual breakdown, degeneration and diseases (Douglas *et al.,* 2001). Different areas of joints, in particular the articular cartilage are subject to different types of loading, such as low-level constant loading during weight bearing, intermittent loading during locomotion and very high impact loading during training or racing in animals (Palmer and Bertone, 1996).

Anatomy of articular cartilage

Articular cartilage composition and thickness vary from joint to joint and the tissue is typically composed of water (75 per cent to 80 per cent), having extracellular matrix collagen II around (50 per cent to 73 per cent) and proteoglycan (15 per cent to30 per cent) respectively. Collagen in articular cartilage especially Collagen type II is mainly consisting of insoluble tightly woven fibers of 30 to 200 nm in diameter. Water and proteoglycan are dispersed through the collagen fibrils and assist in resistance to compression (Maroudas, 1980).

Articular cartilage is composed of four major layers or zones i.e., Zone 1 (lamina splendens) and is the superficial zone. Zone 2 (intermediate or transitional zone/ middle zone). Zone 3 (radial and deep zone). This zone contains the highest content of aggrecan (Maroudas *et al*., 1980) and the lowest content of decorin and biglycan (Poole *et al*. 1996). Zone 4 (deep zone) is partly calcified and so called as calcified zone, and the calcified layer is distinguished by a boundary with noncalcified cartilage called the tidemark (Fig. 1).

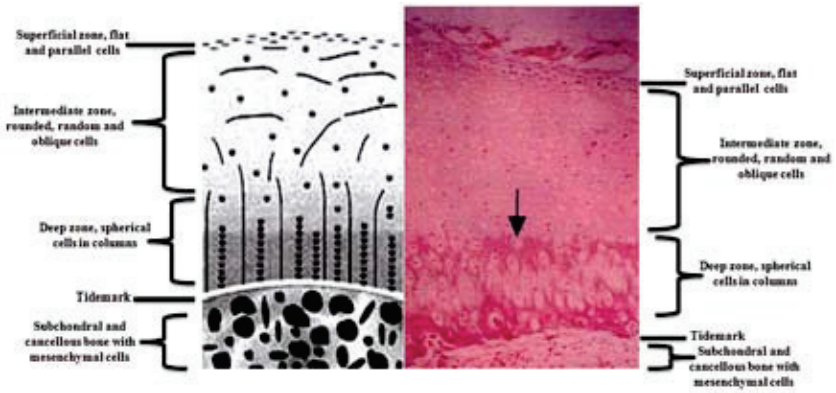

Fig. 1. Understanding the different layers of articular cartilage schematically and histologically.

Injured physiology of articular cartilage

The general response to injury in any tissue is generally divided into three distinct phases *viz*., necrosis, inflammation and repair. As the articular cartilage in adults possesses neither a blood supply nor lymphatic drainage and neural supply so the cartilage follows the necrotic phase soon after the injury. However, there won't be major cell death due to hypoxia as they are less sensitive to lack of oxygen. Although these cells are capable of active synthesis of DNA and increased matrix synthetic activity, the task becomes formidable due to small number of cells with limited potential for metabolic activity (Hirotani *et al.,* 1975). It is, therefore, important to note here that only if damage extends through the basal layers of the cartilage to the vascular subchondral cortex, all three phases of repair becomes possible.

Wounds that are limited to the cartilage itself, without penetration of the subchondral bone (Shapiro *et al*., 1993), stimulate brief cell replication and matrix turnover. Cartilage regeneration gain more scores (Fig. 3: International Cartilage Research Society based scaling) only when the cells from the marrow elements come forward to fill the cartilage defect with new tissue, cartilage injury penetrates beyond subchondral bone and the surrounding are inundated with sufficient growth factors such TGF ß1, IGF-I &II, FGF or EGF (Shapiro *et al*., 1993; Singh *et al*., 2007). The extent to which the new tissue resembles articular cartilage depends on the age and species of the host as well as the size and location of the defect and also based on the presence of type II collagen and aggrecan (Fig. 6). However, complete restoration of the hyaline articular cartilage and the subchondral bone to a normal status is rarely seen, and, till date, no concrete treatment has been shown to be predictable in this regard.

Matrix metabolism

Chondrocyte and synovial cells respond to a variety to cytokines and growth factors that stimulate the production of destructive proteinases. Matrix Metalloproteinase (MMPs) are neutral zinc endoproteinases that collectively degrades all the components of the ECM (Woessner and Nagase, 2000) and have received considerable attention with respect to arthritic tissue destruction because their expression correlates strongly with collagen degradation (Price, 1999). ADAM (for 'a disintegrin and metallo proteinase') belongs to the family of proteinases, are also expressed in cartilage (McKie, 1997), however their roles in tissue maintenance and destruction are still unclear. Recently, ADAMTS (ADAM with thrombospondin motifs) proteins (subset of ADAM family)

have been reported to specifically cleave aggrecan (Kuno, 2000). Although these metalloproteinases are not MMPs, recent evidence indicates that TIMP-3 is a potent inhibitor (Kahiwagi, 2001). Cysteine proteinases (cathepsin B and L, which can degrade cartilage and bone) have been seen in Reumatoid Arthritis synovial lining (Keyszer, 1998) and causes cartilage degradation (Creemers, 1998). Cathepsin K has also been to be pathological in bone and cartilage resorption (Saftig, 2001). Serine proteinases, particularly those associated with the plasmin cascade, have been implicated in tissue destruction (Van der Lann, 2000).

Impact of MMPs in the pathological destruction of cartilage gets accelerated further by various pro-inflammatory cytokines that perturbs the balance between synthesis and degradation of extracellular matrix (ECM)s. Rapid loss of proteoglycan has been documented as compared to collagen depletion following pro-inflammatory cytokine release (Cawston, 1998). Degradation of pro-inflammatory cytokines occurs through lysomal phagocytosis process of fibroblasts (Everts, 1996). This phagocytic phenomenon is highly important for cartilage restoration and therefore studies to unfurl this rare event are required. Whether chondrocytes possess this function is still unclear, however CD44-mediated endocytosis and breakdown of hyaluronate has been reported earlier in chondrocytes (Culty *et al*., 1992), suggesting the differences in mechanisms of ECM breakdown among mesenchymal cells. Cytokines and growth factors have been subdivided into pro-inflammatory anti-inflammatory effects. In arthritis, these generally exhibit either catabolic or anabolic effects on cartilage, respectively (Fig. 2).

Anti-inflammatory cytokines

The effect of anti-inflammatory cytokines include the promotion of matrix synthesis and repair (Smith, 2000), induction of protective enzyme inhibitors such as Tissue inhibitor metalloproteinase (TIMPs) (Hui *et al*., 2001), downregulation of destructive enzymes and reduction in the levels of pro-inflammatory cytokines (Smith, 2000).

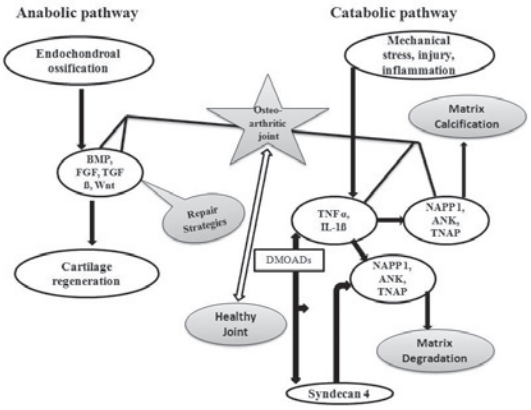

Fig. 2. Primary factors responsible for balancing the anabolic and catabolic pathways of the arthritic joint in animals and humans.

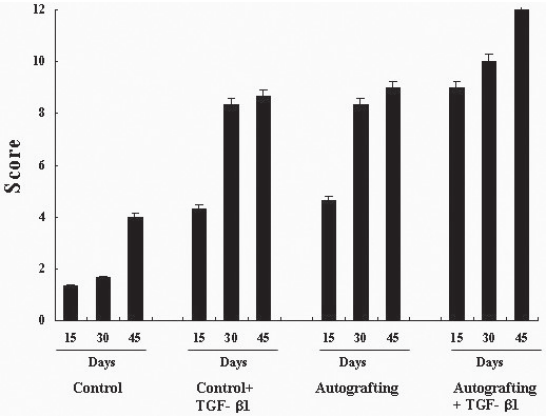

Fig. 3. Assessment score of cartilage repair based on ICRS assessment scale

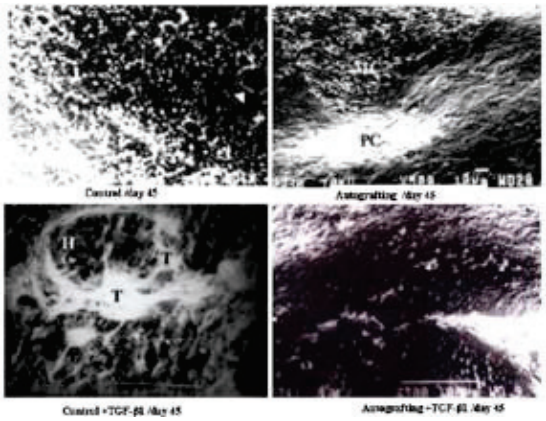

Fig. 4. Surface electron microscopic evaluation of articular cartilage repair Control: Cells showing circular arrangements (arrows) towards the defect.

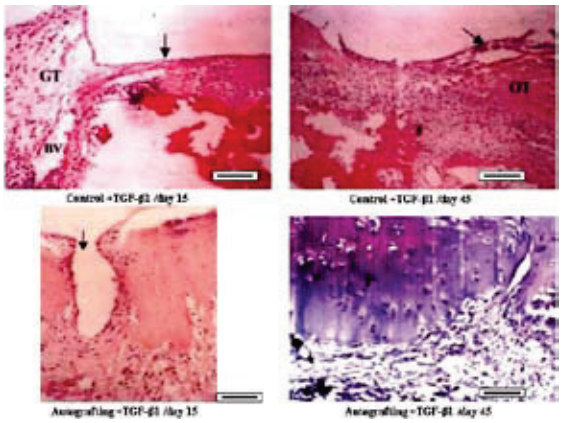

Fig.5. Histological observations of articular cartilage during repair process.

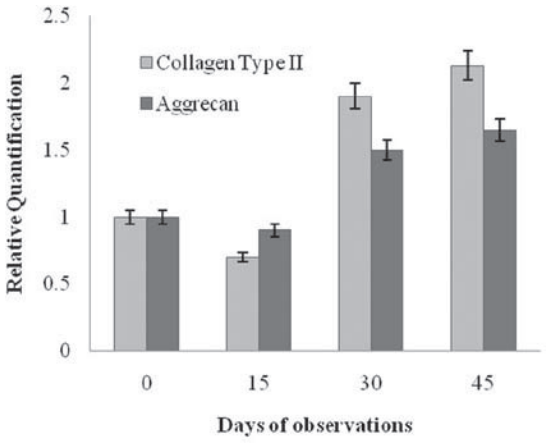

Fig.6. Quantitative real-time PCR for the chondrocyte specific cell type marker gene during healing of full thickness defect with osteochondroal autograft.

TGF-β has a major role in chondroprotective mechanisms and is known to promote matrix synthesis. Cartilage contains vast stores of TGF-β, as much as 300-500 ng/g of cartilage, with most of it in the latent, inactive form that requires proteolytic processing (Moraler, 1994). Various hypotheses have been put forward to explain the physiological significance of this large excess, and although the mechanism of TGF-β autocrine regulation is still not understood, the presence of such reserves strongly implies a role in cartilage metabolism and a key role for TGF-β activation and proteoglycan production (Fig. 5). A recent report, however, has indicated that TGF-β might also cause synovial hyperplasia and osteophyte formation (Bakker, 2001) (Fig. 5).

IGF-1 is also present in elevated levels in the cartilage, which appears to have a role in stimulating chondrocyte anabolic activity (Fig. 2). This growth factor is sequestrated from its cell-surface receptor in the ECM by IGF-binding proteins (IGFBPs) and fibronectin (Martin and Buckwalter, 2000), this complex system is termed as IGF-1 axis, which tightly regulates the bioavailability of IGF-1. IGFBP-3 expression appears to increase with age, paralleling an age-related decline in matrix synthesis, and is also overexpressed in osteoarthritic cartilage where it could cause result from damage –induced IGF-1 release from Insulin like growth factor-Binding Protein (IGFBP) pools (Singh *et al*., 2007). An age-related failure in this system could thus contribute to degenerative disease and gives some credence to the belief that osteoarthritis is due to the wear and tear of old age. The protective effects of IGF-1 have been further demonstrated using adenoviral vectors that specifically result in its overexpression (Martin and Buckwalter, 2000).

Hyaluronan is a ubiquitous component of the extracellular matrix and occurs transiently in the cell nucleus and cytoplasm. It promotes cell motility, adhesion and proliferation and has an important role in morphogenesis, wound repair, and tumor metastasis (Entwistle *et al*., 1996). Cell motility is central to the effect of each of these processes (Fig. 4). Hyaluronan is synthesized actively during wound healing and is an important substrate for leukocyte migration during inflammation (Entwistle *et al*., 1996). Disturbance of regulation of these processes has been alleged to be responsible for errors in morphogenesis, aberrant repair, exaggerated inflammatory responses, and tumorigenesis. Hyaluronan binding, ligand specificity, and stimulation of signal pathways can be modulated by soluble forms of the receptors, by alternatively spliced cell surface isoforms and glycosylation variants of the receptors. It was reported previously that one injection hyaluronan inhibited knee contracture formation approximately 50 per cent in an experimental animal model (Amiel *et al.,* 1985). Hyaluronan is a naturally occurring non-sulfated glycosaminoglycan consisting of alternating N-acetylglucosamine and glucuronic acid residues to form an extended linear polymer. It is also a component of healthy articular cartilage. Hyaluronan is used commonly in surgical procedures by direct application onto tissues, providing a viscous solution that helps prevent tissue dehydration and can reduce improper healing of surgical wounds. It is has been observed to be upregulated during embryonic development and wound healing (Toole, 1997). When hyaluronan combines with collagen scaffolds, this construct

promotes greater cell proliferation synthesis of chondroitin sulfate glycosaminoglycans in vitro when compared to control collagen scaffolds (Kawasaki *et al.*, 1999).

Epidermal Growth Factors are principally found in submaxillary glands and Brunners gland. The primary action attributed to EGF is to promote proliferation of mesenchymal, glial and epithelial cells. EGFs has been well documented to have mitogenic and chemotactic effects on fibroblasts (Brown *et al*., 1989). EGF appears to have little effect on promoting PLF (Periodontal ligament formation) mitogenesis, chemotaxis or matrix synthesis in PLFs. It has been suggested that epidermal growth factor- Receptors (EGF-Rs) may act as a PLF phenotype stabilizer, and reports from invitro studies have concluded that the up-regulation of EGF-R on PLFs is associated with maintaining the cells in an undifferentiated state (with decreased alkaline phosphatase activity); while down regulation of EGF-R is related to the differentiation of cells into osteoblasts or cementoblasts (Matsuda *et al.,* 1993).

Repair biology of articular cartilage

Majority of the mammalian tissues repair with collagen scar, which occurs through DNA replication and cell division. On the contrary, mature chondrocytes do not elicit regeneration as 3H-thymidine autoradiographs of mature articular cartilage failed to show grains over adult cells (Stockwell and Meachim, 1973) though chondrocytes from immature cartilage are capable of mitotic activity (Mankin, 1968). It has been observed and quested occasionally whether the mature cartilage cell "turns off the switch" for DNA synthesis or whether, like the brain cell, it irreversibly "breaks the switch". If the cell at maturity disassembles or permanently injures the DNA replicative apparatus, theoretically it would be unable to respond to any stimulus by active mitosis (including lacerative or mechanical injury. However, analysis of cartilage from joints with osteoarthritis has demonstrated mitotic figures (Hirotani *et al*., 1975) which showed that chondrocytes are capable of mounting a significant reparative response and can replicate their DNA to form new cells.

Chondrocyte's ability also matters as far as the repair process is concern. Ample evidence now exists that articular chondrocytes from immature and adult animals can show either increased or decreased rates of proteoglycan synthesis (Fig. 5) in response to diverse physical and pathological states as osteoarthritis or joint injuries (Mankin, 1974), altered hydrostatic pressure (Kaye *et al*., 1980), varied oxygen tension, alteration in pH (Schwartz *et al.,* 1976), calcium concentration (Palmoski and

Brandt, 1979) , substate concentration , and the presence of growth hormone , ascorbate, vitamin E, cortisol, and so on . Therefore, it is n reasonable to suppose that even injured adult cartilage chondrocytes have the capacity to substantially increase their rate of matrix synthesis, and that the possibility exists of chondrocyte participation in the repair of articular cartilage.

The nature of the repair tissue after subchondral abrasion is variable and range from fibrous to hyaline –like cartilage and it has also been reported that healing improves with continuous passive motion (Harry *et al.,* 1991). Durability of the regenerated cartilage is questionable and not known clearly. Several researchers have observed the nature of the repair tissue to change from hyaline to fibrous cartilage with the passage of time (Buckwalter *et al*., 1988).

The rate of transport of large solutes is thought to depend on convection. The early stages of healing of cartilage are characterized by deposits of a fibrin network within the blood clot and the fibrinous network extends from one end of the defect to the opposite edge, however, it is not well organized in the depths of the defect. At the surface, this fibrinous arcade appears to serve as scaffold to direct the laying down of mesenchymal cell derived from the marrow, which eventually produce a fibrocartilaginous surface zone analogous to the tangential zone of normal articular cartilage. This zone contains flattened chondroblasts with more collagen and less proteoglycan than found in deeper regions of the articular cartilage. The mesenchymal cells between the surface and the deeper marrow differentiate to chondroblasts and synthesize a cartilage matrix, which makes up most of the tissue and is indistinguishable histochemically from normal articular cartilage (Shapiro *et al.,* 1993). The deeper layers of this cartilage undergo a polarized sequence of typical endochondral differentiation, which is then completed by osteoblasts derived from the marrow mesenchymal cells, which form bone on the surface of the calcified cartilage cores (Fig. 5). In the depths of the defect, new intramembranous bone is formed directly, independent of the endochondral sequence, from osteoblasts also derived from marrow mesenchymal cells. In most instances, as the cartilage repair matures, the subchondral region is also modified to form a compact subchondral bone plate, with the deepest layers of the cartilage forming a typical tidemark. Deep to the subchondral plate, trabeculae are organized as cancellous bone, which is initially woven but eventually becomes lamellar. The source of repair cells from the entire sequence is from the undifferentiated mesenchymal cells of the underlying marrow. The absence of effective repair in

partial –thickness defects in which damage is limited to the cartilage and does not traverse the subchondral bone has been well documented (Shapiro *et al*., 1993) and confirms indirectly the importance of the marrow as a source of repair cells.

Modern repair modules for articular cartilage

Scaffolds: Recently scaffolds have been used for the repair of articular cartilage lesions. It is expected from scaffolds that they must fulfill specific requirements with regards to biocompatibility, endurance and their structural stability. In addition, they have to be able to induce maturation and differentiation of the cellular structures which they need to support. Three dimensional scaffolds have demonstrated a high grade cellular adherence and mechanical stability, and few other properties that make them very effective. Scaffolding materials are generally of either natural or synthetic origin. The most common natural materials are hyaluronan and collagen based matrices that simulate the natural articular cartilage. Hyalograft C is a hyluronan based scaffold which has been used clinically in the treatment of articular cartilage lesions (Pavesio *et al.,* 2003). Experimental scaffolds with type I and II collagen—glycosaminoglycan matrices (Breinan *et al.,* 2001) and hyaluronan-based biodegradable polymer scaffold (HYAFF-11) have been found very encouraging (Grigolo *et al.,* 2002). Polyglycolic and polylactic acid have been used as synthetic scaffolds. In general, synthetic materials are very promising as they prevent immune reaction problems of the natural materials. Nevertheless, many improvements such as adhesion of the graft with the adjacent native cartilage, biomaterials that can be implanted without open joint surgery and cell-seeding with genetically modified cell populations are required in future with highest priorities.

Mesenchymal Stem Cells: Ample number of in vivo studies were performed in the past using mesenchymal stem cells (MSCs) for the repair of articular cartilage because chondrogenic potentials were shown by mesenchymal stem cells during invitro studies (Gao and Caplan, 2003). Biochemical, histological and mechanical properties of articular cartilage have been demonstrated with the use of chondrogenic MSCs in polymeric scaffolds earlier in both in-vitro and in-vivo studies (Lee *et al*., 2004). With this belief, chondrogenic MSCs seeded with biodegradable porous polymeric scaffolds became the treatment of articular cartilage defects with no difference in mechanical and histological properties compared with ACI (Autologous chondrocyte implantation). MSCs are pluripotent stem cells with the ability to differentiate into a variety of other

connective tissue cells, such as chondral, bony, muscular, and tendon tissue. (Gao and Caplan, 2003). Bone marrow-derived MSCs are pluripotent cells that can differentiate among others into osteoblasts, adipocytes and chondrocytes depending on the environmental cues (Singh *et al.,* 2007a). Isolation of these cells are relatively easy and can be obtained from the marrow and then be expanded in culture, keeping the differentiation property in appropriate culture conditions (Pitterger *et al*., 1999). Recently, based upon a specific CD marker expression, an MSCs population, that is located in normal, as well as in the osteoarthritic human articular cartilage was recognized (Pitterger *et al.,* 1999). However, there is need to further studying the ability of these MSCs to clone and expand in vitro.

Embryonic Stem Cells: ESCs have been found to have huge regenerative potentials for cartilage regeneration (Thomson *et al*., 1998). However, teratoma formation and ethical concerns are some of the issues which normally confront ESCs use as repair module for cartilage (Wakitani *et al.,* 2003; Shiwani *et al.,* 2011). On the contrary, no teratoma's have been reported to form with these cells when used within the scaffolds (Nakajima *et al*., 2008), therefore proper optimization of donor cells to differentiate as well as inhibit tumor growth may help to mitigate the concerns of potential teratoma formation. Moreover, ethical issues related to the use of human embryonic stem cells can be circumvented only with the development IPS cells (induced pluripotent stem cells) just by forcing the somatic cells (Yu *et al*., 2007; Takahashi *et al.,* 2007) to express four genes encoding the transcription factors Oct-4, Sox-2, Nanong, and Lin-28. Yet at another instance, Yamanaka and colleagues generated induced pluripotent stem (iPS) cells, capable of germline transmission, from adult human dermal fibroblasts with the four factors: Oct3/4, Sox2, Klf4, and c-Myc (Takahashi *et al.,* 2007). Human iPS cells were similar to human ES cells in morphology, proliferation, surface antigens, gene expression, epigenetic status of pluripotent cell-specific genes, and telomerase activity. Furthermore, these cells could differentiate into cell types of the three germ layers in vitro and in teratomas. These findings demonstrate that iPS cells can be generated from adult human fibroblasts. However, these cells generated tumors in animal models, thus further improvement of gene delivery techniques are needed in order to prevent mutation leading to tumor formation (such as retroviral integration). Another significant advance in the field of embryonic stem cell research has been made recently by developing human ES cell lines from a single cell harvested from the blastomere, without destroying the embryo

or affecting its development (Klimanskaya *et al.,* 2007). While these research findings are exciting, the techniques are still in their early developmental stage (Doerflinger, 2008). The formation of three-dimensional tissue by differentiation of such ES cells has not yet been demonstrated. Therefore, more scientific data are needed for the acceptance hESCs in approaches of cartilage regeneration.

Adult Stem Cells: In contrast to ES cells, adult stem cells transplanted in preclinical animal models have shown no evidence of tumor formation, not even, teratomas. This may be due to fact that ESCs and adult stem cells are under different epigenetic control and miRNA regulatory profile. Nevertheless, these properties, combined with the possibility of autologous transplantation, demonstrate significant advantages over embryonic stem cells in many proposed clinical applications in the current time (Singh *et al.*, 2007). There are several types of adult somatic stem cells with different potentials to differentiate toward functionally mature cells. Adult somatic cells with the potential to differentiate into mesenchymal lineages such as cartilage, bone, ligament, tendon, fat (Singh *et al.*, 2007 b) and other connective tissues have been referred here as mesenchymal stromal cells [MSCs] despite the different nomenclature found in the literature to refer to dissimilar cells with similar properties. MSCs obtained from different adult tissue sources are found to have dissimilar capacities to differentiate to mature cells. Adipose derived MSCs have a higher adipogenic potential while cells from the periosteum exhibit a superior osteogenic and chondrogenic ability. In addition, periosteal MSCs exhibit high osteogenic potential while also exhibiting chondrogenic and myogenic capacity (Singh *et al.,* 2007c). Though skeletal muscle derived MSCs are known for their relatively low potential for chondrogenesis they do possess multi-differentiation capacity. Synovial membrane forms the lining of the chondyle surface and it is the most proximal vascularized tissue to cartilage. MSCs derived from the synovial membrane and synovial fluid show high chondrogenic potential which is comparable to that of bone marrow derived MSCs. It is assumed that these cells originate from the bone marrow and migrate to the synovium via vasculature. Interestingly, some studies have also indicated the presence of MSC - like progenitor cells in the surface zone of normal and osteoarthritic adult human articular cartilage as well as in immature bovine articular (Hiraoka *et al.,* 2006) cartilage (Mareddy *et al.,* 2007). This observation is intriguing because it shows lack of regeneration of diseased articular cartilage in apparent presence of chondrogenic cells. A probable explanation

is that the MSCs found in cartilage are actually recruited from the synovial membrane as a reparative response to damage. This could also explain the detection of a higher number of MSCs in OA cartilage compared to healthy cartilage. However, the increased frequency of progenitor cells in OA-cartilage could also result from proliferation of resident progenitor cells (Alsalameh *et al.,* 2004). These observations incite the presumption that the mere presence of MSCs at the site of injury is not sufficient for induction of repair processes. More likely, MSCs require cues from the microenvironment to differentiate towards chondrocytes.

Adipose Derived Adult Stem (ADAS): Adipose Derived Adult Stem (ADAS) cells are MSC like cells isolated from adipose tissue by collagenase digestion (Gimble and Guilak, 2003; Zuk *et al*., 2001; Wickham *et al*., 2003). Cells of ADAS have been demonstrated to have decreased chondrogenic potential compared to bone marrow-derived MSCs which clearly suggests that the original location of the tissue appears to play a significant role in determining their differentiation potential. ADAS have been shown to have in vitro and in vivo chondrogenic potential. Cartilage-like tissue was observed in pellet cultures and cell-loaded carriers (agarose, alginate & gelatin sponge) after treating ADAS with TGF-ß and dexamethasone. Cartilage formation was also observed in vivo after implanting the cells in an alginate carrier into subcutaneous pockets in immunodeficient mice, and repairing articular cartilage in a rabbit model (Betre *et al*., 2006; Dragoo *et al.,* 2007). Defects repaired with these cells were filled with hyaline cartilage that integrated with the surrounding host tissue. ADAS cells and MSCs isolated from the same patient were when compared showed no significant differences as far as yield of adherent stromal cells, growth kinetics, cell senescence, multi-lineage differentiation capacity, and gene transduction efficiency were concerned (De Ugarte *et al*., 2003). ADAS cells are abundantly found in the adipose tissues and have a potential similar to bone marrow-MSCs which could be used in tissue engineering applications and as gene delivery vehicles.

Bone Marrow Stromal Stem Cells: Existence of bone marrow stromal stem cells was first established by Cohnheim (1867). Later in the mid-1970's Friedenstein and colleagues performed a series of detailed in vitro and in vivo studies describing the isolation and properties of fibroblast-like cells, termed marrow stromal cells, which adhered to the surface of the cultured dish in whole bone marrow samples (Friedenstein, 1992). These cells proliferated, formed colonies, and showed osteochondrogenic capacity, even after passaging the cells in culture over many population doublings.

Later on Caplan and colleagues called the process of marrow stromal cell proliferation and differentiation mesengenesis and termed the cells mesenchymal stem cells (Haynesworth *et al*., 1992). However, marrow stromal cells and mesenchymal stem cells represent a heterogeneous population of cells with different properties. Prockop and colleagues isolated demonstrated several subpopulations of marrow stromal cells with different self-renewal, differentiation, and engraftment capacity (Colter *et al.,* 2000; Prokop *et al.,* 2001). Developmentally immature marrow MSCs, reminiscent of ES cells, termed marrow-isolated adult multilineage inducible (MIAMI) cells have been isolated using a unique method and these cells were later characterized by a defined molecular profile and a broad differentiation potential (D' Ippolito *et al.,* 2006). Multipotent adult progenitor cells (MAPCs) have also been isolated using different strategies with similar immature stem cells population having strong self renewal and differentiation capacity (Reyes *et al.,* 2002). BM-MSC subpopulations, considered to be of mesodermal origin, have been found to differentiate, under appropriate conditions, to cells types of tissues derived from other germ layers (D' Ippolito *et al.,* 2004). Studies on the chondrogenic differentiation of BM-MSCs have served as the basis to further characterize in vitro the cytokines that regulate chondrogenesis, the signaling pathways activated, and the transcription factors that are involved in the process originally identified using developmental biology approaches. Such findings have further unfurled the roles of sonic hedgehog (Shh), FGF (2, 4, 8, and 10), TGF ß superfamily (1, 3 & BMP-2, 4, 7, and 14), Wnt (3a and 7a) during chondrogenic differentiation and FGF-18 and PTHrP during chondrocyte hypertrophy (Djouad *et al*., 2006). Maintenance of chondrocyte proliferation and inhibition of hypertrophy was demonstrated with the action of Nkx3.2 which was further related to the mechanisms leading to the inhibition of Runx2 (Park *et al.,* 2007). Thus, BM-MSCs, and in particular the more primitive subpopulations, appear to be the most suitable cell source for cartilage repair and tissue engineering approaches, because these cells can be carefully and sequentially directed to progress toward the chondrogenic pathway in a controlled fashion, without the risk of tumor formation, by providing the right molecular cues at the right time. Furthermore, these cells could be molecularly controlled to promote or prevent hypertrophy depending on the therapeutic role or tissue engineer need. BM-MSCs have been the most widely used cells in cartilage repair; alone or in combination with scaffolds such as collagen sponges, hyaluronic acid hydrogels, and

other biomaterials, in several animal models. Articular cartilage is structurally a complex tissue whose function depends partly on the mechanical support of subchondral bone (Radin *et al.,* 1986). Thus, functional restoration of articular cartilage will depend on the mechanical and structural support of subchondral bone tissue.

CONCLUSION

Lately, research has turned to producing a tissue with hyaline cartilage properties. In addition, the ability of this new tissue to consolidate in the native surrounding articular cartilage is a necessity. Production of a hyaline cartilage that will fill the articular cartilage defect may be accomplished with the combination of autologous cultured cells and the appropriate scaffolds. Arthroscopic techniques with the use of tissue engineering products, like Hyalograft C, are very promising. In mosaicplasty, refinements in arthroscopic procedures and instruments may minimize the marginal damage of the transplanted cartilage and may lead to better consolidation with the surrounding healthy articular cartilage. Tissue engineering constitutes a fast developing area in medicine. Useful tools may be pluripotent mesenchymal stem cells, embryonal stem cells, resorbable biomaterials, growth factors and gene transfers. Their combination may eventually lead to the desired outcome in the treatment of articular cartilage defects.

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