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RESEARCH ARTICLE

ARTICULAR CARTILAGE TISSUE ENGINEERING WITH PLASMA-RICH IN GROWTH FACTORS AND STEM CELLS WITH NANO *SCAFFOLDS*

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ABSTRACT

The ability to heal soft tissue injuries and regenerate cartilage is the Holy Grail of musculoskeletal medicine. Articular cartilage repair and regeneration is considered to be largely intractable due to the poor regenerative properties of this tissue. Due to their low self-repair ability, cartilage defects that result from joint injury, aging, or osteoarthritis, are the most often irreversible and are a major cause of joint pain and chronic disability. However, current methods do not perfectly restore hyaline cartilage and may lead to the apparition of fibro- or continue hypertrophic cartilage. The lack of efficient modalities of treatment has prompted research into tissue engineering combining stem cells, *scaffold* materials and environmental factors. The field of articular cartilage tissue engineering, which aims to repair, regenerate, and/or improve injured or diseased cartilage functionality, has evoked intense interest and holds great potential for improving cartilage therapy. Plasma-rich in growth factors (PRGF) and/or stem cells may be effective for tissue repair as well as cartilage regenerative processes. There is a great promise to advance current cartilage therapies toward achieving a consistently successful approach for addressing cartilage afflictions. Tissue engineering may be the best way to reach this objective via the use of stem cells, novel biologically inspired scaffolds and, emerging nanotechnology. In this paper, current and emergent approach in the field of cartilage tissue engineering is presented for specific application. In the next years, the development of new strategies using stem cells, in *scaffolds*, with supplementation of culture medium could improve the quality of newly formed cartilage.

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INTRODUCTION

For a long time, cartilage has been a major focus of the whole field of tissue engineering, both because of the constantly growing need for more effective options for joint repair and the expectation that this apparently simple tissue will be easy to engineer.[1] Articular cartilage has no or very low ability of self-repair and untreated lesions may lead to the development of osteoarthritis (OA).[2] OA is one of the most debilitating joint diseases. No treatment is available. Transforming growth factor β-1 (TGFβ-1) plays a major role in bone and cartilage development and is an important regulator of chondrocyte proliferation and differentiation.[3] One method that has been proven to result in long-term repair of isolated lesions is autologous chondrocyte transplantation. However, first generation of these cells' implantation has limitations, and introducing new effective cell sources can improve cartilage repair. Articular cartilage provides a resilient and compliant articulating surface to the bones in diarthrodial joints. It protects the joint by distributing loads applied to it, so preventing potentially damaging stress concentrations on the bone. At the same time it provides a low-friction-bearing surface to enable free movement of the joint. Articular cartilage may be considered as a visco- or poro-elastic fiber-composite material.[2] Injuries to articular cartilage are one of the most challenging issues of musculoskeletal medicine due to

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the poor intrinsic ability of this tissue for repair. The lack of efficient modalities of treatment for large chondral defects has prompted research on cartilage tissue engineering combining cells, *scaffold* materials and environmental factors.[4] A definite need exists for effective methods to stimulate cartilage regeneration and integration, and provide a durable, long-lasting replacement for the original cartilage. In response to this need, novel bioengineering approaches to induce and enhance cartilage regeneration are being developed. It is well established that cartilage formation begins with mesenchymal condensation leading to chondrogenic differentiation of mesenchymal cells. Then, a dense matrix is produced, serving as the cartilage anlage, a template for the subsequent generation of both the articular cartilage and the subchondral bone.[1] Scientists and engineers have attempted to develop biological grafts for treating cartilage defects by (*i*) cell therapies that recapitulate precartilaginous mesenchymal condensation and stimulate/orchestrate regeneration of cartilage, (*ii*) biomaterial matrices designed to restore important functions of articular cartilage and serve as a template for regenerative turnover, or (*iii*) tissue-engineered grafts for implantation that resemble mature cartilage and have the capacity to integrate with the surrounding tissues.[5,6] Cells are the driving force of cartilage formation and the continual maintenance of the tissue. Cell therapies utilize the implantation of externally cultivated cells to replicate and stimulate native regeneration. Mature chondrocytes were the first cells that found clinical application for cartilage regeneration. The use of mature chondrocytes is based on the premise that native, mature cells are best suited to guide regeneration.[1]

Stem cells are a relevant source of information about cellular differentiation, molecular processes and tissue homeostasis, but also one of the most putative biological tools to treat degenerative diseases.[7] Primarily due to their favorable properties, and despite the current debate, mesenchymal stem cells (MSCs) are increasingly studied and utilized to treat cartilage defects and osteoarthritis.[8] Importantly, MSCs are a heterogeneous cell population that can generate fibrocartilage and hypertrophic chondrocytes along with the desired articular-cartilage-producing chondrocytes.[9] Because of the seemingly quiescent nature of cartilage, its avascularity, and its low cell density, a significant branch of research has been directed towards developing biomaterials that can mimic cartilage matrix and restore function at the defect site.[10] The incorporation of cells into biomaterial *scaffold*s makes cartilage repair more complex, but can significantly help orchestrate regeneration and overcome some of the limitations of using cells or biomaterials alone. In general, tissue engineering combines cells and biomaterial scaffolds into a tissue construct, and then uses engineered control of the construct environment, *in vitro* and *in vivo*, to replicate the native cartilage environment and produce viable grafts for cartilage regeneration. [11] For therapeutic application of tissue engineering, engineered tissue is grown either within a patient or outside the patient and subsequently transplanted into the patient [12] or for experimental application is grown either within an animal or outside the animal and subsequently transplanted into an animal articular cartilage defect model (fig. 1). Figure 1 provides a schematic representation of the process of tissue regeneration in cartilage tissue engineering. Cells are harvested from the animal model and after *in vitro* cell culture; cells are seeded onto *scaffolds* with medium containing chemical stimuli, such as growth factors and differentiation-inducing factors.

Scaffolds fabricated by the electrospinning technique, are three-dimensional (3D) matrices that support cellular growth processes, such as cell adhesion, migration, proliferation, and differentiation, by which cells are colonized onto the *scaffold*. The cell-colonized *scaffold* is then implanted into the animal model, to regenerate bio-compatible, immunocompatible, and biofunctional tissues inside the animal model body. Cells and *scaffolds* are essential to regenerate new tissues with tissue engineering. Cells become the primary component of the engineered tissue and the *scaffold* provides cells with an appropriate physical and chemical environment where they can attach to the surface of the *scaffold*, migrate through the *scaffolds*' pores, and then proliferate.

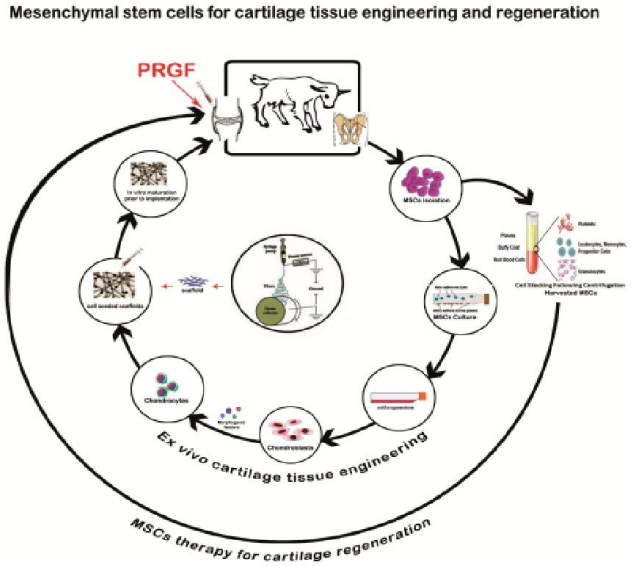

Figure 1. A schematic representation of the process of tissue regeneration in cartilage tissue engineering Use of MSCs as cell therapy for cartilage tissue repair and regeneration

The two potential approaches of MSCs-based cartilage repair and regeneration are illustrated in fig. 1. The 1st is *in vivo* cartilage regeneration via MSCs therapy using its anti-inflammatory and immunosuppressive effects. The 2nd is *ex vivo* cartilage tissue engineering, in which a replacement tissue is constructed *in vitro* using MSCs combined with *scaffold* under appropriate environmental stimuli. As shown in this figure, MSCs are expanded and injected locally into the affected joint. MSCs, due to their potential regenerative functions as indicated, will help to influence the microenvironment to aid in the regeneration of the cartilage. The ease of isolation and expansion and the multipotential differentiation capacity, especially the chondrogenic differentiation property of MSCs, make MSCs the cell type of choice for articular cartilage tissue engineering that aims to replace and regenerate the diseased structure in joint diseases. In addition, their immunomodulatory and anti-inflammatory functions make MSCs the ideal candidate for cell therapy to treat diseases with inflammatory features such as those encountered in OA and Rheumatoid Arthritis (RA). Therefore,

MSCs are actively being considered as candidate cells for the treatment of arthritic joint diseases both as a structural substitute and as a stand-alone cell therapy or as a combination thereof (Fig. 1).

OA is the most common type of arthritis. Its clinical manifestations include joint pain and impairment of movement, and surrounding tissues are often affected with local inflammation. The etiology of OA is not completely understood; however, injury, age, and genetics have been considered among the risk factors. OA is a progressively debilitating disease that affects mostly cartilage, with associated changes in bone. Cartilage has limited intrinsic healing and regenerative capacities. Current pharmacologic treatment for early OA has seen limited success, and various surgical procedures, are able to relieve pain temporarily but eventually fail. Due to the increasing incidence of OA and the aging population coupled with inefficient therapeutic choices, novel cartilage repair strategies are in need.[13]

The availability of large quantities of MSCs and their potential for ready chondrogenic differentiation after prolonged *in vitro* expansion have made MSCs the most hopeful candidate progenitor cell source for cartilage tissue engineering. MSCs loaded on a 3-D *scaffold* under appropriate differentiation cues can undergo chondrogenic differentiation, and the resulting construct can be used as a replacement tissue for cartilage repair (Fig. 1). *In vitro* cartilage tissue engineering in addition to being used for structural replacement of cartilage, MSCs have been used directly in cell therapy for OA cartilage repair *in situ*. OA is associated with progressive and often severe inflammation. For tissue engineering or cell therapy to be successful, measures must be taken to control such an inflammatory environment. Because MSCs have been shown to possess anti-inflammatory function, they are also a suitable cell type for this purpose. Several characteristics of MSCs make them attractive in this respect. First, MSCs have been shown to be able to migrate and engraft onto multiple musculoskeletal tissues, especially sites of injury, and undergo site-specific differentiation. More importantly, MSCs can exert significant effects on local environment and resident endogenous tissue progenitor cells through direct or indirect interactions and soluble factors. In addition, MSCs have shown potent anti-inflammatory and immunosuppressive activities. Taken together, these properties make MSCs a promising candidate for cell therapy for diseases that often involve the immune system, such as RA and OA (Fig. 1).

MATERIALS AND METHODS

The objective of our prospective pilot ongoing research is to explore the role that implanted MSCs may play in tissue repair or regeneration of the injured joint, by delivery of an autologous preparation of stem cells with nano *scaffolds* and PRGF to caprine knee joints following induction of OA. Our pilot study employed PRGF on MSCs with nano *scaffolds* in goat injured joint to highlight the regenerative effect of MSCs therapy on the injured knee. We present an experimental treatment that involved concentrating the blood in a centrifuge to prepare PRGF and injecting them back into the damaged joint.

Adult stem cells were isolated from caprine bone marrow, expanded in culture, implanted with a poly (lactic-*co*-glycolic) acid (PLGA) *scaffold*, added to PRGF, and then injected in the injured knee to test their effect. OA was induced unilaterally in the knee joint of donor animals. After 6 weeks, a single dose of 10 million autologous stem cells were suspended in a diluted solution of sodium hyaluronan which was then delivered to the injured knee by direct intraarticular injection. Control animals received sodium hyaluronan alone**.**

PRGF Preparation

PRGF was prepared by Bassuet et al method.[14] Blood, was collected by vacutainer system in plain sterile tubes, each containing Acid Citrate Dextrose (ACD) (Spectra Group Company). Then the tubes were first centrifuged at 200 g for 15 minutes and plasma was separated in plain sterile tubes by sterile syringes. The tubes containing the plasma underwent a second centrifugation at 1200g for 10 minutes. The supernatant containing platelet poor plasma was discarded by a sterile syringe, keeping only some plasma at the bottom of each tube. The platelet pellet was re-suspended in the plasma left. Finally PRGF was collected by a sterile syringe in one plain sterile tube (Fig. 2). Just before injection, $CACl_2$ (10%) (Improve diagnostics) were added to the prepared 5 ml PRP (50 µl for each 1 ml PRP) by sterile syringe to get PRGF, then activated PRGF was rapidly injected into the pre-sterilized knee by the classic lateral approach using 22-g needle.

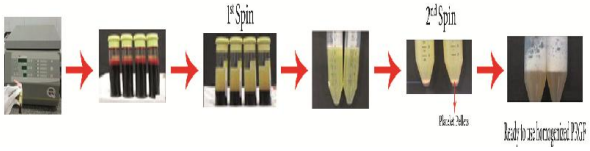

Figure 2. Steps of preparation of Plasma-rich in growth factors

Table 1. Isolation of mesenchymal stem cells

Isolation Protocol	Laboratory Investigations of Interest of Isolated Cells
▪ Mononuclear cell separation from bone marrow on ficoll. ▪ Primary culture of mononuclear cells. ▪ Subculture of isolated MSCs: The cells from primary culture were seeded for the 1st and 2nd passage.	▪ Counting and viability testing of isolated cells by the trypan blue exclusion test. ▪ Flow cytometry using mesenchymal stem cell markers for the expression of CD73 and CD44 and lack of expression of CD34.

Preparation of Caprine MSCs

A heparinized bone marrow aspirate (3–7 ml) is obtained from the iliac crest of castrated male Nobian Brees goats. Briefly, nucleated cells were isolated with a density gradient (Ficoll-Paque; GE HealthCare, Waukesha, WI) and resuspended in culture medium; Delbecco's Modified Eagle's Medium (DMEM), supplemented with 10% fetal bovine serum (FBS) and 1% penicillin-streptomycin (10,000 µg/mL). Cells were incubated at 37 °C in 5% CO2 for ~14 d. Culture medium was changed every 2 to 3 d. When cells reached 80% to 90% confluence, cultures were washed twice with phosphate-buffered saline (PBS), and the cells were trypsinized with 0.25% Trypsin in 1 mM EDTA for 5 min at 37 °C. After

centrifugation, cells were resuspended in medium and subcultured for ~10 d. MSCs in culture were characterized by their adhesiveness and fusiform shape and by flowcytometry MSCs surface markers (CD44+, CD73+ and CD34-) as shown in Table 1.[15-20]

Flowcytometric analysis of BM-MSCs

BM-MSCs ($1x10^6$) were incubated with 500 µl of 2% FBS/PBS in flowcytometer specific tubes at 4°C for 30 min with 10 µl of MSCs antibody specific for CD44, CD73 and CD34. On the day of implantation, cells were trypsinized, collected, and resuspended in PBS in sterile tubes. BM-MSCs on nano *scaffold* were mixed with the PRGF, and the mixture was left to gel. Briefly, the preliminary results of our ongoing pilot research showed that the isolated cells possessed the basic features of MSCs (fig. 3). Most markers were expressed in PLGA group one week earlier than in the control group. A significant improvement was observed in the functional knee movement of animal models with osteochondral defects who were reconstructed with BM-MSCs transplanted on PLGA nano *scaffolds* enriched with PRGF.

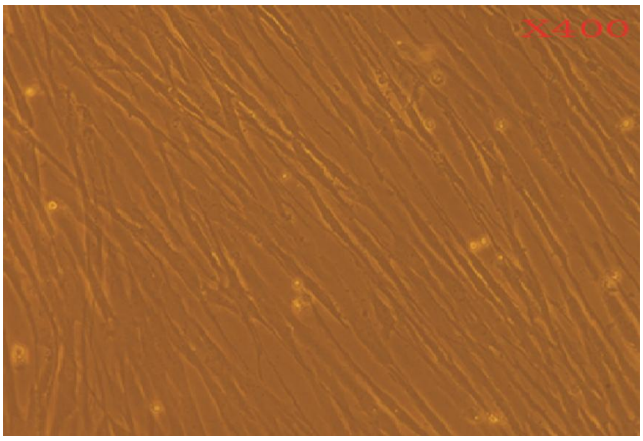

Figure 3. Morphology of bone marrow mesenchymal stem cells examined under the inverted microscope Representative photomicrograph shows fibroblastoid cells with about 80-90% confluences

Articular cartilage and Chondrocytes

Composition of Adult Articular Cartilage

Articular cartilage is hyaline cartilage covering the surfaces in diarthrodial joints and is 2 to 4 mm thick. Unlike most tissues, it does not have blood vessels, nerves, or lymphatics. It is composed of a dense extracellular matrix (ECM) with a sporadic distribution of highly specialized cells called chondrocytes. The ECM is primarily composed of water, proteoglycans, and collagen, with other noncollagenous proteins and glycoproteins present in lesser amounts.[21,22] Together, these components help to keep water within the ECM, which is crucial to maintain its unique mechanical properties. Articular cartilage consists of 2 phases: a fluid phase and a solid phase. Water is the major component of the fluid phase, contributing up to 80% of the wet weight of the tissue. Inorganic ions such as calcium, sodium, chloride, and potassium are also found in the fluid phase. The solid phase consists mainly of the ECM, which is porous and permeable, as well as chondrocytes.[23,24] Chondrocytes, account for less than 5–10% of the total tissue volume.[25] Mature chondrocytes are totally encapsulated in the dense cartilage ECM and are not able to migrate or proliferate in a significant manner, unlike cells in bone, thus potentially limiting the regenerative capacities of cartilage after injuries. However, ECM, which includes several organic constituents like collagen, proteoglycans, and other noncollagenous proteins, accounts for most of the dry weight of the tissue.[26]

Mechanical characteristics and compressive properties of articular cartilage

Articular cartilage is a specialized thin connective tissue with unique viscoelastic properties that increases joint congruence, it protects the subchondral bone from high stresses, and it reduces friction at the edge of long bones.[27] Its main function is to provide a smooth, lubricated surface for low friction articulation and to facilitate the transmission of loads to the underlying subchondral bone.[28] The relationship between proteoglycan aggregates and interstitial fluid provides compressive resilience to cartilage through negative electrostatic repulsion forces.[29] With increasing age, there is reduction in the hydration of the matrix, with a corresponding increase in compressive stiffness. This may have implications for the underlying subchondral bone, which may face increased forces as the cartilage loses its ability to undergo reversible deformation.[30]

Repair properties of articular cartilage

Although articular cartilage is composed of a small percentage of chondrocytes, a dense extracellular matrix (ECM) prevents chondrocyte mobility. Moreover, articular cartilage lacks vascular, lymphatic, and neural networks, as well as various local progenitor cells. It has also been described as having high levels of protease inhibitors, which may inhibit efficient tissue repair.[31,32] Cartilage lesions are therefore usually irretrievable, due to its avascular nature and consequent lack of access to a pool of potential reparative cells and growth factors.[33] Osteochondral lesions, which involve both the articular cartilage and the subchondral bone, usually lead to the formation of fibrocartilage which has completely different biomechanical properties from the native hyaline cartilage and does not protect the subchondral bone from further degeneration. This process involves both repaired and adjacent native tissues which lead to the insurgence of severe pain, joint deformity, and loss of joint motion.[34]

Articular cartilage defects

Articular cartilage defects, which are due to traumatic destruction or degenerative joint diseases, are principally divided into two categories: partial-thickness and full-thickness cartilage defects.[35,36] The partial-thickness defects only damage the zonal articular cartilage but do not penetrate the underlying subchondral bone, rendering the defect site inaccessible to bone cells, blood cells, and progenitor cells in bone morrow. Thus, the defect site lacks fibrin clots and other self-healing responses. Although some enzymatic and metabolic activities occur and chondrocytes may begin to proliferate and synthesize ECM just after the creation of a

partial-thickness defect, there are still not enough new chondrocytes to migrate into the injured sites to effectively repair the injury. Furthermore, the reparative activities of chondrocytes typically cease before healing of the cartilage defect, thus resulting in a lasting defect that decreases tissue function and can serve as a starting point for tissue degeneration.[36] Full-thickness (or osteochondral) defects penetrate the entire thickness of articular cartilage, past the calcified zone, and into the subchondral bone. Full-thickness defects are accessible to blood cells, macrophages, and mesenchymal progenitor cells,[36] all of which are involved in a spontaneous immune response and a healing process after injuries.[37] Immediately following injury, the defect void is filled with a fibrin clot and an inflammatory response is activated. Thereafter, mesenchymal stem cells from bone marrow migrate into the defect, gradually replacing the fibrin clot and completely filling the defect after one week.[36] Many of these MSCs can later differentiate into chondrocytes, which secrete a proteoglycan-rich ECM and repair the damaged cartilage tissue. However, fibrous tissues with weaker mechanical properties and higher permeability are formed in defect sites.[38] Consequently, in full-thickness defects, the spontaneous repair process is only transient and imperfect, and tissue degeneration eventually takes place several months later and proceeds continuously.[36,37] Then the cartilage tissue often becomes hypertrophic and is finally replaced by the progressive deposition of subchondral bone.[35,36] At this point, while chondrogenesis may still occur sporadically, complete resurfacing is seldom observed, leading to bone to bone articulation, inflammation, significant pain, and disability.

Chondrocytes

The chondrocyte is the only cell type within articular cartilage. Despite their presence throughout the tissue, chondrocytes occupy less than 10% of the total volume. Each chondrocyte is surrounded by its ECM, has few cell-to-cell contacts, and relies on diffusion for nutritional support. The chondrocyte shape and size varies depending on its zonal position. The superficial cells are ellipsoidal and are aligned parallel to the surface. The transitional cells are spherical and are randomly distributed. The deep cells form columns aligned perpendicular to the tidemark and the calcified zone. Chondrocytes are derived from mesenchymal cells. Their primary function is to maintain the ECM, the component of articular cartilage that provides its unique material properties. Chondrocytes rarely divide after skeletal growth is completed. The chondrocyte is metabolically active and able to respond to environmental stimuli and soluble mediators, including growth factors, interleukins, and certain pharmaceuticals. They are responsive to mechanical loads, hydrostatic pressure changes, osmotic pressure changes, as well as injury and degenerative arthritis.[39]

Plasma-rich in growth factors

PRGF is also known as platelet rich plasma (PRP), but since the role of growth factors has been stressed, therefore we prefer to refer to it as PRGF. PRGF is included within the field of regenerative medicine.[40,41] PRGF is an autologous concentration of human platelets contained in a small volume of plasma.[42,43] PRGF is now in common use during oral and maxillofacial surgery, as it is believed to enhance the integration of periodontal implants and accelerate the repair process.[44,45] PRGF has also been reported to provide significant improvements in the healing of complex wounds.[46] PRGF has been used to treat musculoskeletal injuries in both people and horses, where it is applied via an open surgical approach or closed percutaneous injection.[40,47] The main advantages of PRGF include its availability, affordability, and minimally invasive harvest, since it is produced from the patient's own blood after collection by simple venipuncture.[48] PRGF is safe with no serious complications.[49]

Platelet Growth Factors

The main growth factors contained in the platelet alpha-granules are: platelet derived growth factor (PDGF) in the isoforms AA, BB and AB, beta transforming growth factor (TGF- β1 and TGF- β2), vascular endothelial growth factor (VEGF), basic fibroblastic growth factor (bFGF), epidermal growth factor (EGF), insulin- like growth factor (IGF-1, IGF-2 and IGF-3), fibroblast growth factor (FGF) and others.[50-52]

PDGF

PDGF is the main growth factor in platelets, as it is the first to initiate activity at the site of injury and it directs revascularisation and the synthesis of collagen and osteogenesis.[53] The release of PDGF in the wound bed has a chemotactic effect on monocytes, neutrophils, fibroblasts, mesenchymal stem cells and osteoblasts. It is also a powerful mitogenic factor for fibroblasts and smooth muscle cells and it participates in the three phases of the healing cascade, particularly angiogenesis, formation of fibrous tissue and re epithelialization.[54]

The TGF-βs

The TGF-β family consists of over than 35 members and it includes, beside the TGF-βs, activins and bone morphogenetic proteins (BMPs).[55] Activins participate in the regulation of several biological processes, including cell differentiation and proliferation.[56] Bone morphogenetic proteins (BMPs) are a group of growth factors originally discovered by their ability to induce the formation of bone and cartilage, now they are considered as a group of signals orchestrating tissue architecture throughout the body.[57] The most commonly found in PRGF are TGF- β1 and TGF- β2. These are growth factors linked to both healing of connective tissue and the regeneration of bone tissue. The β1 structure is found in a great quantity in platelets, lymphocytes and neutrophils, while the β2 is encountered in osseous extracts, platelets and neutrophils.[53,58] It is active during inflammation and it influences cell migration, proliferation and replication. In cartilage devolpment, it regulates chondrocyte proliferation and differentiantion[52] as well as the connection between fibronectins. The most important functions of TGF- β1 and TGF- β2 are chemotaxis and mitogenesis.[54]

Transforming Growth Factor Beta 1 or **TGF-β1**

TGF-β1 is a polypeptide member of the transforming growth factor beta superfamily of cytokines. It is a secreted protein that performs many cellular functions, including the control of

cell growth, cell proliferation and apoptosis.[59] Most of the cells secrete TGF β as a large latent complex, which then binds to the extracellular matrix (ECM) to provide a ''controlled release'' of the growth factor to its target cells. This process requires the release from the ECM and then cleavage for activation of the growth factor, which normally occurs by proteolytic or mechanical means.[60,61] Platelets are a major source of TGF- β1 in the circulation as they release latent growth factor in response to activation, in contrast to other cellular sources of this growth factor; the TGF β contained in platelets is secreted in an active form upon release from the alpha granules, and this characteristic may have implications for TGF β as delivered by PRGF treatment.[62] In normal physiologic states, TGF β-1 is generally considered to exert anti-inflammatory and immunosuppressive effects.[63] It influences cell migration, proliferation and replication, as well as the connection between fibronectins.[54] It strongly induces granulation tissue formation by attracting fibroblasts and stimulating collagen production and angiogenesis.[64] The main activity of TGF β-1 is synthesis and preservation of the extracellular matrix.[65] In orthopedic tissues, TGF β-1 plays a crucial role by functioning as a potent regulator of chondrocyte proliferation and differentiation, and of extracellular matrix deposition.[66] TGF β-1 is required for cartilage matrix homeostasis and intrinsic repair.[67-69] TGF-β-1 causes chondrogenic induction of mesynchymal stem cells (MSCs).[70] TGF-β-1 is one of the many signaling factors that are involved in chondrogenesis[71], in the maintenance of the chondrocyte phenotype, and in the homeostasis of articular cartilage.[72] The loss of the potential for the formation of cartilage-like tissue by expanded chondrocytes can be recovered by exogenous TGF-β.[73] TGF β-1 effects on bone are contradictory as well; it induces matrix production and proliferation in osteoblasts.[74] TGF β-1 supplementation of demineralized bone matrix has been shown to accelerate the repair process.[75] TGF β-1 is important for the maintenance of normal tendon integrity and repair.[76] Successful therapeutic use of TGF β, either as a lone agent or as a component of PRP, will require the ability to select the desired TGF β effects on matrix production and quality without incurring pathologic fibrosis.[76]

Plasma-Rich In Growth Factors And Mesenchymal Stem Cells

Tissue engineering employs a combination of methods offered both by cellular biology and bioengineering, using cells alone or combined with different biomaterials to partially or completely substitute or regenerate tissues or damaged organs, thereby restoring their structure and function.[77] Among the cells utilized, stem cells deserve highlighting because when they are stimulated by specific signals, they are capable of auto-renewal and auto-differentiation in various cellular types with specialized functions. The possibility of inducing this differentiation, relative to the location to be treated, is an excellent therapeutic alternative for obtaining more efficient tissue regeneration.[78] The main sources for obtaining the described stem cells are through embryonic and hematopoietic lineages. Cells of embryonic origin, although totipotent, are difficult to manipulate, can undergo terato-carcinogenesis and pose ethical questions that remain an important barrier to their use.[79] Bone marrow and umbilical cord blood, apart from being hematopoietic sources for stem cells, are also considered to be excellent sources for MSCs and, although not totipotent, are capable of differentiating themselves into cells of other lineages. Studies have demonstrated that MSCs present a capacity for differentiation into cells of mesodermic lineage (bone, fat, cartilage and tendons) and those of endo- and ectodermal lineages, being able to develop into cardiomyocytes, neurons and hepatic cells, among others. They also possess an immunomodulatory capacity.[80] Studies have demonstrated that MSCs derived from adipose tissue possess identical characteristics to those obtained from bone marrow and umbilical cord blood; however, they are easier to obtain.[80] The technique to acquire the stem cell is less invasive and less painful than aspirating a bone marrow sample from the hip or the sternum. There are up to 1,000 times more stem cells in a gram of fat than in a gram of bone marrow. *In vitro* studies have demonstrated that MSCs have their differentiation controlled by specific growth factors.[20,81-88] PRGF allows for an adjunctive autologous value added to the stem cell fraction. PRGF is derived from an autologous whole blood sample from the patient and once processed yields many healing growth factors. PRGF can help with survival of the cells upon introducing to the patient, reduce inflammation as well as provide cyto-protective properties when transit is necessary. Therefore, because of its high concentration of growth factors, PRGF has been used to induce the proliferation and differentiation of MSCs and, consequently, increase the capacity of these cells for tissue regeneration.[89] In addition to the positive effects on MSCs proliferation, differentiation and migration, PRGF may also provide a three-dimensional substrate for cell seeding by virtue of the presence of fibrinogen, which gives rise readily to fibrin gel upon thrombin or calcium activation.[90]

Effect of preparation-rich in growth factors on adult and bone marrow-derived Mesenchymal stem cells

As candidate cells applicable for tissue engineering-based approaches to cartilage repair, MSCs have noticeable advantages over chondrocytes due to their abundant availability, robust chondrogenic activity accompanied by cartilage matrix production, and multi-lineage differentiation ability to repair osteochondral defects.[68,91,92] Among their various tissue sources, MSCs derived from bone marrow (BMSCs) are employed most extensively in cartilage engineering. Many researchers have found that PRGF exhibits a mitogenic effect on MSCs.[93-97] When human BMSCs in monolayer were cultured with 10% inactivated autologous PRGF, a fivefold increase in cellular proliferation was seen at day 7 relative to the control without PRGF supplementation.[98]

Effect of preparation-rich in growth factors on cartilage repair in animal models *In vivo*

In another study, PRGF was used as a bioactive scaffold alone or with MSCs to fill osteochondral defects (4 mm in diameter, 3 mm in depth) in the femoropatellar groove of rabbits. At 12 weeks post-implantation, PRGF scaffold alone yielded better macroscopic, histological and immunological results than those in the untreated group, but worse than those of the PRGF -MSC group. [99] In light of the poor mechanical properties of PRGF, Sun and colleagues[100] used PRGF as an additive to poly (lactic-*co*-glycolic acid) (PLGA) *scaffolds* to repair large

osteochondral defects (5 mm in diameter, 4 mm in depth) in rabbits. After 4 and 12 weeks, compared to PLGA alone, PRGF increased the content of cartilaginous ECM, and improved subchondral bone formation. These results were consistent with those from a previous study that used bilayer collagen scaffold with or without PRGF to repair osteochondral defects (4 mm in diameter, 3 mm in depth) in rabbits.[101] PRGF contains growth factors that stimulate cellular anabolism, inflammatory mediators and modulators that exert anti-inflammatory effects and fibrinogen that acts as a biomaterial *scaffold*. Regenerative medicine and tissue engineering offer the exciting potential of developing alternatives to total joint replacement for treating articular cartilage injuries and OA. Research findings derived from basic and preclinical studies and from clinical trials collectively suggest that PRGF is a promising treatment for cartilage injuries and relieving symptoms owing to its three known biological properties. Firstly, PRGF has an anabolic effect on chondrocytes, MSCs and synoviocytes with resultant increases in cell proliferation, cartilaginous ECM accumulation, and hyaluronic acid HA secretion. Secondly, PRGF may act as a bioactive cell *scaffold* to fill defects and enhance cartilage regeneration. Thirdly, PRGF has the potential to inhibit inflammation and alleviate OA symptoms with a clinically acceptable safety profile.[102]

Mesenchymal stem cells

Mesenchymal stem cells (MSCs) are a heterogeneous subset of stromal cells that can be isolated from bone marrow, marrow aspirates, skeletal muscle, adipose tissue[103], synovium and many other connective tissues.[104] Adult MSCs were originally isolated from bone marrow in 1999 by Pittenger and co-workers[105], who demonstrated their multilineage differentiation potential or multipotency. Subsequent studies have identified the presence of stem cells in a number of adult tissues, including adipose tissue, muscle, dermis, periosteum, synovial membrane, synovial fluid and articular cartilage. Due to their culture-dish adherence, they can be expanded in culture while maintaining their multi-potency.[106] They can differentiate into cells of the mesodermal lineage, giving rise to a range of specialized connective tissues including bone,[107-109] adipose tissue,[110,111] cartilage,[106,109,110], intervertebral disc,[112-114] ligament [113-115] and muscle.[110] MSCs in culture can be induced to generate chondrocytes, myocytes, adipocytes, osteoblasts and tenocytes.[105,116] Some studies have demonstrated that MSCs can interact with immune cells, leading to the modulation of a number of effects or functions.[116] The immunomodulatory properties of MSCs may be exploited for the treatment of inflammatory and rheumatic conditions.[117] MSCs can promote tissue repair and the survival of damaged cells.[116] After *in vivo* administration, MSCs can induce peripheral tolerance and migrate to injured tissues where they have the capacity to exert immunosuppressive properties[118] and inhibit the release of pro-inflammatory cytokines and promote the survival of existing cells and the repair of damaged tissue.[116] They are being clinically explored as a new therapeutic tool for treating a variety of immune-mediated diseases.[119] MSCs show considerable promise for use in repairing and rebuilding damaged or diseased mesenchymal tissues.[106] MSCs have potential applications in tissue engineering and regenerative medicine and may represent an attractive option for bone, cartilage, tendon and ligament regeneration. However, it is not clear which adult tissues MSCs should be sourced from. There are currently several different types of MSCs that have been proposed as potential sources of cells for cartilage repair: bone marrow-derived MSCs, adipose tissue-derived MSCs, synovial membrane-derived MSCs and Wharton's jelly/umbilical cord-derived MSCs. The best choice of cell type for cartilage repair will depend on its availability and chondrogenic differentiation potential.[120]

Nanotechnological advances in cartilage repair

Mature hyaline cartilage has a very low self-repair potential due to its intrinsic properties. For this reason, researchers have focused in the search of methods to reproduce the tissue characteristics of hyaline cartilage and induce complete cartilage repair. A new approach for the treatment of articular cartilage defects is the use of biocompatible *scaffolds*.[121] It has been demonstrated that the natural materials used in the field of cartilage tissue engineering could potentiate the production of collagen type II and sulfated glycosaminoglycans by both chondrocytes and stem cells.[122] Instead, synthetic materials present more easily molding characteristics, relatively easy production and the ability to control dissolution and degradation.[123] The synthetic materials most widely used are poly (a-hydroxy acids), especially poly (lactic acid) (PLA), poly (glycolic acid) (PGA) and their co-polymers poly-lactic-*co*-glycolic acid (PLGA), poly (ε-caprolactone) (PCL), poly (propylene fumarate) and polyethylene glycol (PEG).[124,125] ECM of cartilage tissue is comprised of collagen and proteoglycans which are nanometers in scale. Thus *scaffolds* for cartilage tissue engineering have to be accomplished on the nanoscale to achieve similar mechanical and physical properties to native tissue. To better recapitulate the ECM environment for cartilage tissue engineering, researchers have introduced several biological signals, including chondroitin sulfate (CS), hyaluronic acid and collagen, into tissue-engineered *scaffolds*. Recently, nanofibrous *scaffolds* composed of poly (vinyl alcohol) (PVA), a hydrophilic synthetic biodegradable polymer, and chondroitin sulfate, have been shown to enhance tissue formation *in vitro* and also *in vivo*, when these were implanted into rat osteochondral defects.[126] In addition, combination of PVA-PCL electrospun nanofiber *scaffolds* with MSCs showed improvement of tissue healing compared to those which received cell-free *scaffolds*, suggesting their potential as a suitable graft for articular cartilage reconstruction.[127-131]

Conclusion

The use of MSCs for orthopedic conditions is an active area of research. Tremendous advances have been made in our understanding of the versatility of MSCs and their intrinsic capacity to differentiate into multiple cell lineages. Demands of the field of tissue engineering have intensified the effort to understand the potential therapeutic value of MSCs. The ability to understand the fundamental cell and molecular biology of MSCs will help attempts to manipulate these cells towards specific therapeutic goals. While an ideal recipe for cartilage regeneration is yet to be formulated, we believe that it will contain cell, biomaterial, and tissue engineering

approaches, blended into an effective method for seamless repair of articular cartilage. We conclude from this study that the transplantation of autologous culture-expanded BM-MSCs in PRGF with nano *scaffolds* shows a great promise in the treatment of articular cartilage defects. Further researches in this field can provide a way for advancement of application of cartilage tissue engineered-MSCs in regenerative medicine.

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