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Cell Therapy and Tissular Engineering to Regenerate Articular Cartilage

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1. Introduction

Osteoarthritis (OA) is a degenerative joint disease characterized by deterioration in the integrity of hyaline cartilage and subchondral bone (Ishiguro et al., 2002). OA is the most common articular pathology and the most frequent cause of disability. Genetic, metabolic and physical factors interact in the pathogenesis of OA producing cartilage damage. The incidence of OA is directly related to age and is expected to increase along with the median age of the population (Brooks, 2002).

The capacity for the self-repair of articular cartilage is very limited, mainly because it is an avascular tissue (Mankin, 1982; Resinger et al., 2004; Fuentes-Boquete et al., 2008). Consequently, progenitor cells in blood and marrow cannot enter the damaged region to influence or contribute to the reparative process (Steinert et al., 2007).

There are a lack of reliable techniques and methods to stimulate growth of new tissue to treat degenerative diseases and trauma (Wong et al., 2005).

Modalities of cellular therapy to repair focal articular cartilage defects include the implantation of cells with chondrogenic capacity (Koga et al., 2008) and creating access to the bone-marrow. Of the numerous treatments available nowadays, no technique has yet been able to consistently regenerate normal hyaline cartilage. Current treatments generate a fibrocartilaginous tissue that is different from hyaline articular cartilage. To avoid the need for prosthetic replacement, different cell treatments have been developed with the aim of forming a repair tissue with structural, biochemical, and functional characteristics equivalent to those of natural articular cartilage (Fuentes-Boquete et al., 2007). This review summarizes the options for treatment of articular cartilage defects from both the experimental and clinical perspective (Fig. 1).

2. Perforation of the subchondral bone

This treatment is one of the most popular marrow-stimulating techniques based on the principle of inducing invasion of mesenchymal progenitor cells from the underlying subchondral bone to the lesion site, in order to initiate cartilage repair (Pelttari et al., 2009). This minimally invasive procedure has a low cost and is currently being used as the first treatment in patients not treated of cartilage defects. When the defect affecting the cartilage

penetrates to the bone and bone marrow spaces (osteochondral injury), mesenchymal cells from the bone marrow migrate with the hemorrhage and remain in the blood clot filling the defect, and are differentiated into articular chondrocytes thus been responsible for the repair of the defect (Fig. 2) (Shapiro et al., 1993). The opening of subchondral vascular spaces is utilized for several surgical strategies, such as arthroscopic abrasion (Friedman et al., 1984), subchondral drilling (Muller & Kohn, 1999), spongialization (Ficat et al., 1979) and microfracture (which produces the best results) (Steadman et al., 1999). In most cases, bone is formed in the bony defect and fibrocartilaginous tissue is formed in the chondral lesion (Johnson, 1986; Buckwalter & Mankin, 1998). In the case of large osteochondral defects, the ability to spontaneously repair the damage is negligible. On the contrary, if the chondral defect is small, articular cartilage can be completely repaired in full. The critical size of the lesion so that it will self-repair remains unknown.

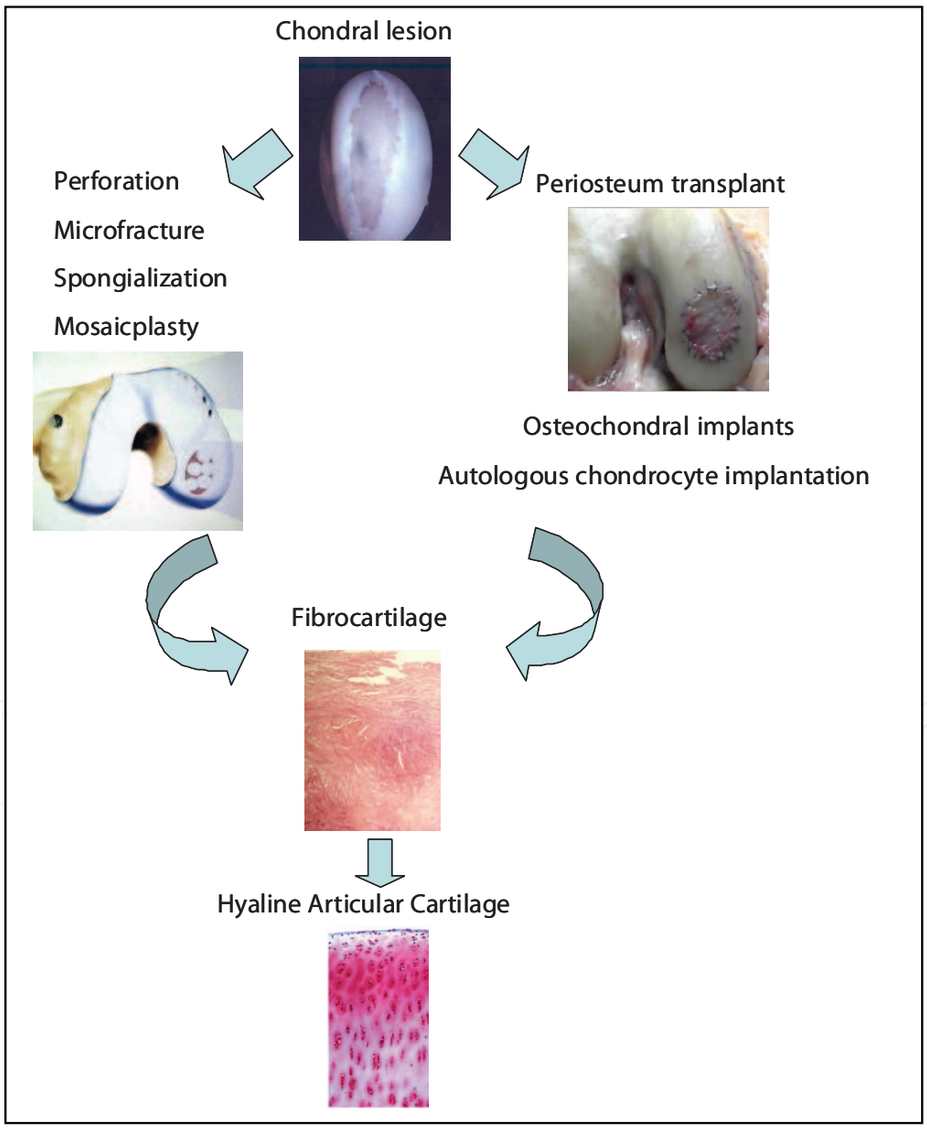

Fig. 1. Different treatments of articular cartilage defects.

The outcome of these procedures is highly variable and frequently results in repair tissue composed of fibrocartilage with some limitations in quality and duration as compared to native hyaline cartilage (Pelttari et al., 2009). Experimental studies in rabbits (Metsaranta et al., 1996; Menche et al., 1996) and dogs (Altman et al., 1992) have shown that the repair tissue generated by these processes is fibrocartilaginous in nature, differing from hyaline articular cartilage in biochemical composition, structural organization, durability and biomechanical properties, and degenerates over time (Shapiro et al., 1993; Menche et al., 1996). In addition, the newly formed subchondral bone is thicker than the native subchondral bone (Qiu et al., 2003). The co-expression of types I and II collagens in repair tissue does not occur until one year following subchondral penetration (Furukawa et al., 1980). Clinical results, to some degree, contradict the findings relating to the quality of the repair tissue. For example, the treatment of knee osteochondral defects by microfracture has provided good clinical results after two years (Knutsen et al., 2004). This longevity, however, seems to be age-dependent, with the most persistent repair cartilage in patients under the age of 40 (Kreuz et al., 2006a). Although the initiation of a degenerative process for tissue repair has been described at 18 months after microfracture (Kreuz et al., 2006b), and 7 to 17 years after microfracture, improvement in articular function and pain relief were preserved (Steadman et al., 2003).

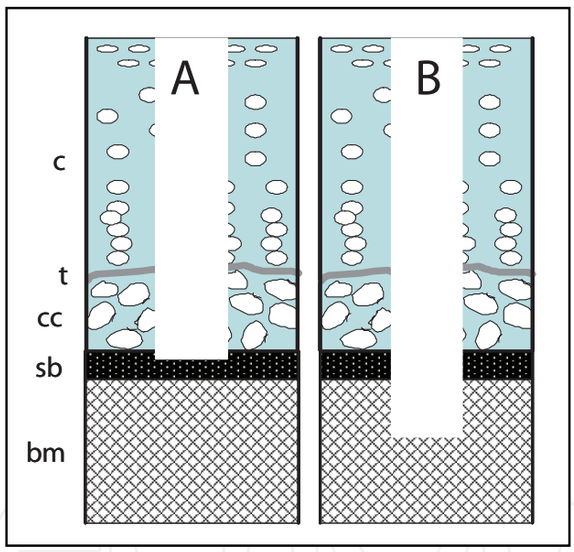

Fig. 2. Types of articular cartilage defects. In a partial defect the lesion includes cartilage tissue and part of the subchondral bone [A]. In a deep defect the lesion extends to the bone marrow [B]. C, uncalcified articular cartilage; t, tidemark; cc, calcified articular cartilage; sb, subchondral bone; bm, bone marrow.

3. Implants of periosteum and perichondrium

Tissue grafts have potential benefits since they allow the introduction of a new cell population embedded in an organic matrix, and reduces the development of fibrous adhesions between the articular surfaces before forming a new articular surface.
Periosteum and perichondrium contain mesenchymal stem cells (MSCs) that are capable of chondrogenesis (O´Driscoll et al., 2001; Duynstee et al., 2002). In particular, periosteum

consists of a fibrous outer layer, containing fibroblasts; and an inner layer or cambium, in direct contact with the bone, of higher cellular density, which contains MSCs.
Experimental studies in rabbits, indicated that the grafts of periosteum and perichondrium produce an incomplete filling of the chondral defect, and showed no significant differences between the two grafts in the quality of the repair tissue (Carranza-Bencano et al., 1999). In contrast, in a horse model, it was observed that chondrogenesis was more frequent and of greater magnitude in the grafts of periosteum than in perichondrium (Vachon et al., 1989). In both cases, these membrane implants forms a fibrocartilaginous repair tissue that does not seem to mature over time (Dounchis et al., 2000; Trzeciak et al., 2006). However, the clinical effects of a perichondrium implant are similar those of subchondral perforation. At 10 years following either procedure there were no significant differences observed between their outcomes (Bouwmeester et al., 2002). However, the graft of perichondrium requires an additional intervention.
With age, decreases the chondrogenic potential of periosteum, decreasing the ability of MSCs to proliferate and differentiate into chondrocytes (O´Driscoll et al., 2001). This procedure has confirmed the improvement of joint function and pain relief (Korkala & Kuokkanen, 1995). The periosteum has the advantage of being readily available for transplantation. However, the technique of obtaining and management of periosteum is a critical step and determining the chondrogenic potential; if the cambium layer is not preserved, the procedure fails (O´Driscoll & Fitzsimmons, 2000).
At present, there is no sufficient evidence to justify the use periosteum and perichondrium implants in the treatment of chondral defects.

4. Osteoperiosteal implants

The cylinder of bone graft covered with periosteum has been used for the treatment of osteochondral defects. Although it has been reported that its clinical application produces improved joint function and pain relief (Korkala & Kuokkanen, 1995), studies in animals show a neosynthesized tissue with fibrous features (van Susante et al., 2003). When the graft is accompanied by chondrogenic inductors it acquires a fibrocartilaginous appearance (Jung et al., 2005). Also, bleeding from bone marrow spaces from the injury probably interferes with the repair action of the periosteum germ layer. In fact, in a rabbit model of osteoperiosteal implant it was found that nearly 67% of repair tissue cells were derived mainly from the bone marrow (Zarnett & Salter, 1989).
Osteochondral grafts have the advantage of providing matrix and viable chondrocytes that maintain this matrix (Czitrom et al., 1990; Schachar et al., 1992; Ohlendorf et al., 1996). In addition, it is possible to retrieve the subchondral bone and the contour of the joint of patients with osteochondral defects or articular incongruity. The articular cartilage transplantation as part of an osteochondral graft provides the decrease in joint pain (Beaver et al., 1992), perhaps by the replacement of the innervated area of the subchondral bone by a graft without innervation.

5. Mosaicplasty

Autologous mosaicplasty is considered to be a promising alternative for treatment of small to medium-sized focal chondral and osteochondral defects (Bartha et al., 2006). This technique involves the translocation of osteochondral cylinders, or plugs, from a low-

weight-bearing normal site to a high-weightbearing diseased site. The injured area is completely covered by means of the combination of different sizes of cylinders (Szerb et al., 2005). The donor sites spontaneously repair with mesenchymal stromal cells from the bone marrow to promote a new fibrocartilaginous tissue.

This procedure, which clinical application started in 1992 (Hangody & Karpati, 1994; Hangody et al., 2001) is considered a promising alternative for the treatment of chondral and osteochondral defects of small and medium-size load in synovial joints (Bartha et al., 2006). However, it is limited by several factors. The ideal diameter of the defect should range between 1 and 4 cm^2. In addition, clinical experience shows that age is a limiting factor, it is recommended to apply this technique only for patients under 50 years. Contraindications to the use of mosaicplasty include infection, tumor and rheumatoid arthritis (Szerb et al., 2005).

Arthroscopic evaluations at 5 (Chow et al., 2004) and 10 years (Hangody & Fules, 2003) after osteochondral cylinder implantation showed survival of the transplanted articular cartilage, congruency between opposing (treated and untreated) joint surfaces and fibrocartilaginous repair of the donor sites. However, if the osteochondral cylinders protrude above the surface, joint problems can arise. At 4 months post-surgery, patients with protruding cylinders experienced a "catching sensation" and some of these patients reported joint pain. Arthroscopic examinations of these cases revealed fissures in the osteochondral cylinders and fibrillation around the recipient site (Nakagawa et al., 2007).

The use of autologous mosaicplasty is limited by the defect size, which determines the number of osteochondral cylinders required. Thus, in large defects the best option is osteochondral allogenic transplantation. In addition, the implanted tissue comes from an area of low load, showing a thin thickness, a different histological structure and, therefore, a lower functional capacity for dealing with charge absorption.

The articular cartilage produced by this technique exhibits topographical variations in morphological, biochemical and physical properties (Xia et al., 2002; Rogers et al., 2006). Because the implanted tissue is harvested from a low-weight-bearing area, the cartilage is thinner and differs in histological structure from cartilage from high weight-bearing areas (Fragonas et al., 1998; Gomez et al., 2000).

6. Osteoarticular allotransplantation

Due to the avascular nature of chondrocytes and the fact that they are encapsulated in the extracellular matrix (ECM), articular cartilage is considered a privileged immunological tissue (Langer & Gross, 1974). Thus, the allogenic transplant may be the solution for problems arising from the autologous mosaicplasty (avoiding injury to the low load zone of cartilage, can produce a large number of osteochondral cylinders and these can come from the same load area). In fact, osteochondral allograft in knee has shown a good integration and provides a functional improvement at 2 years (McCulloch et al., 2007), showing a 85% of implant survival after more than 10 years after intervention (Gross et al., 2005).

7. Autologous chondrocyte implantation

A cell-based therapeutic alternative offering more effective repair of focal articular cartilage defects is autologous chondrocyte implantation (ACI) which was developed in a rabbit experimental model (Grande et al., 1987 & 1989). The first clinical application of this method

was performed by the group of Brittberg (Brittberg et al., 1994), which also demonstrated the successful repair of articular cartilage in rabbits transplanted with autologous chondrocytes (Brittberg et al., 1996). Currently the autologous chondrocyte implantation is a safe and effective therapeutic alternative to repair focal articular cartilage lesions (Pérez-Cachafeiro et al., 2010; Brittberg et al., 1994; Richardson et al., 1999; Peterson et al., 2000; Roberts et al., 2001). This procedure is also used for patients with osteochondritis dissecans (Peterson et al., 2002), but not for osteoarthritis joints. Because the results of this technique are highly age-dependent, the use of this procedure is recommended for patients younger than 55 years of age. The technique involves obtaining, by arthroscopy, articular cartilage explants from low-weight-bearing areas. Chondrocytes are then isolated and expanded *in vitro* to obtain a sufficient number of cells (approximately 10-12x10^6 cells) to introduce into the defect site, where they are expected to synthesize new cartilaginous matrix. In a second surgical intervention, the periosteum of the patient is removed from the proximal extremity and sutured to the edge of the cartilage injury, guiding the cambium layer towards de defect. This will close the defect cavity to retain the suspension of chondrocytes. Then, chondrocytes of the patient are resuspended in a liquid medium and injected into the cavity. A recent study assessed the efficacy and safety of ACI in 111 patients and demonstrated good clinical results in about 70%of the cases after 3 to 5 years (Pérez-Cachafeiro et al., 2010). Sometimes these autologous articular chondrocytes are introduced into the defect site as a cell suspension or in association with a supportive matrix (matrix-assisted ACI, MACI) (Pelttari et al., 2009). MACI uses a cell-seeded collagen matrix for treatment of cartilage defects. A prospective clinical investigation carried out in 38 patients with localized cartilage defects for a period of up to 5 years after surgery, showed that MACI represents a viable alternative for treatment of local cartilage defects of the knee (Behrens et al., 2006).

The outcome of these chondrocyte-based techniques is generally quite good (Minas, 2001; Peterson et al., 2000) but in many cases results in the formation of non-hyaline cartilage repair tissue with inferior mechanical properties and limited durability (Pelttari et al., 2009). ACI has several technical limitations: *a)* obtaining cartilage explants requires an additional surgical intervention, adding to the articular cartilage damage that increases the osteoarthritic process (Marcacci et al., 2002); *b) in vitro* chondrocyte proliferation must be limited because the capacity to produce stable cartilage *in vivo* is gradually reduced when cell divisions are increased (Dell´Accio et al., 2001); *c)* aging reduces the cellular density of the cartilage, which impacts chondrocyte proliferation capacity *in vitro* (Menche et al., 1998) and the chondrogenic potential of the periosteum (O´Driscoll & Fitzsimmons, 2001), *d)* cell culture procedures take too long (3 to 6 weeks) and increase the risk of contamination, *e)* risk of leakage of transplanted chondrocytes from the cartilage defects, *f)* the effects of gravity causing the chondrocytes to sink to the dependent side of the defect, resulting in an unequal distribution of cells that hampers the homogenous regeneration of the cartilage (Díaz-Prado et al., 2010c; Sohn et al., 2002), *g)* not the least the reacquisition of phenotypes of dedifferentiated chondrocytes in a monolayer culture (Kimura et al., 1984; Benya & Shaffer, 1982) and *h)* hypertrophy of tissue (Steinwachs & Kreuz, 2007; Haddo et al., 2004). The use of periosteum membrane poses constraints and the need for wide surgical incision, hypertrophy of the periosteum peripheral implant and its potential for ectopic calcification. As an alternative it has been proposed the use of a membrane collagen type I/III (Haddo et al., 2004; Krishnan et al., 2006; Robertson et al., 2007). The use of both kinds of membranes shows no significant differences in the clinical assessment, although arthroscopic analysis

showed that after implantation of periosteum a substantial number of patients required a cleanup of the peripheral hypertrophy (Gooding et al., 2006).

In 1997, the American Society FDA (Food and Drug Administration) approved the cellular technology that uses autologous chondrocytes to repair articular cartilage lesions in the knee. This was the first type of cellular technology that was regulated by the industry for use in human transplantation (Brittberg et al., 2001).

The first article about ACI in humans appeared in 1994 (Brittberg et al., 1994). Clinical and arthroscopic evaluations of femoral implants showed good results after 2 years and the histological study of biopsies of the new tissue showed a similar appearance to hyaline cartilage in 11 of 15 cases of femoral implant. From this first approach further studies, based on clinical or arthroscopic evaluations, have demonstrated the durability of the implant. Thereby, after 5-11 years of treatment showed good or excellent clinical results in 51 of the 61 patients (Peterson et al., 2002). Histological analysis of the *de novo* formed tissue revealed some heterogeneity in the quality of the repair tissue. Of the 41 biopsies obtained one year following implantation, 10% consisted of hyaline cartilage; 24% consisted of a mixture of hyaline cartilage and fibrocartilage; 61% were entirely fibrocartilage and 5% consisted only of fibrous tissue (Tins et al., 2005).

Other studies at one year after implantation have shown that fibrocartilaginous morphology regions and hyaline morphology regions coexist in the same biopsy; both types having proteoglycans and type II collagen (Richardson et al., 1999; Roberts et al., 2001). Furthermore, aggrecanase activity was higher than metalloprotease activities in the fibrocartilaginous regions although both enzymes were found (Roberts et al., 2001). The expression of type IIA and IIB collagen mRNA was also detected (Briggs et al., 2003). These mRNA expressions seem be characteristic of the prechondrocytic state (type IIA) and differentiated chondrocytes (type IIB) (Nah et al., 2001). These results suggest that ACI induces the regeneration of articular cartilage, probably by the turnover and remodelling from an initial fibrocartilaginous matrix using enzymatic degradation and synthesis of type II collagen (Roberts et al., 2001). It is believed that this process continues for more than 24 months following the implantation (Peterson et al., 2000, Bentley et al., 2003) and takes place in three specific stages: cell proliferation (the first 6 weeks), transition (7 to 26 weeks) and remodeling (beyond 27 weeks) (Minas & Peterson, 1997).

8. Allotransplantation and xenotransplantation of chondrocytes

Other therapeutic alternatives are allotransplantation (Wakitani et al., 1989; Rahfoth et al., 1998; Schreiber et al., 1999) and xenotransplantation of chondrocytes (Fuentes-Boquete et al., 2004, Ramallal et al., 2004), that elude the damage added to the joint during autotransplantation to obtain isolated chondrocytes. Allotransplantation is constrained by the necessity for compatible donors and limitations on storage of cartilage or chondrocytes because cryopreservation reduces survival and proliferation of chondrocytes (Rendal-Vázquez et al., 2001). Xenotransplantation may resolve some of these problems, but this therapeutic alternative has rarely been investigated. The immune barrier is an important objection to the use of both of these therapeutic procedures, although its application in articular cartilage presents fewer difficulties than in other tissues. Even though isolated chondrocytes result in immunogenic reaction, alloimplantation of chondrocytes encapsulated in their ECM (Schreiber et al., 1999) or embedded in collagen gel (Wakitani et al., 1989) or agarose (Rahfoth et al., 1998) resulted in few or no rejection reactions. Notably,

xenotransplantation *in vivo* of cultured pig chondrocytes into rabbit chondral defects closed with periosteal membrane no signs of infiltration by immune cells (Ramallal et al., 2004).

9. Mesenchymal stem cells transplantation

Within the bone marrow stroma, a subset of non-hematopoietic cells referred to as MSCs exists. These cells can be isolated by adherence to plastic, expanded *ex vivo* and induced, both *in vitro* or *in vivo*, to terminally differentiate into multiple mesoderm-type lineages, including osteocytes, chondrocytes, adipocytes, tenocytes, myotubes, astrocytes and hematopoietic-supporting stroma (Barlow et al., 2008; Minguell et al., 2000; Caplan, 1991) and also into cell types of ectodermal (e.g., neurons) and endodermal (e.g., hepatocytes) origin (Pasquinelli et al., 2007). Furthermore, MSCs from different tissue sources can have biologic distinctions. For example, MSCs derived from bone marrow show a higher potential for osteogenic differentiation (Muraglia et al., 2000), while MSCs of synovial origin show a greater tendency toward chondrogenic differentiation (Djouad et al., 2005). Under identical culture conditions for differentiation, MSCs isolated from the synovial membrane show more chondrogenic potential than those derived from bone marrow, periosteum, skeletal muscle or adipose tissue (Sakaguchi et al., 2005). Studies of cartilage injury repair in animal models using MSCs embedded in collagen gel (Wakitani et al., 1989) or injected into defects closed with periosteal membrane (Im et al., 2001) indicate that MSCs can differentiate *in vivo* into a number of cell types in different biologic environments.

This procedure uses cells isolated from small tissue samples, proliferated in culture, to obtain the appropriate number for clinical applications. They can be implanted in the donor patient, obviating rejection problems. MSCs may be a tool for tissue repair that has the advantage of avoiding the problem of immunological rejection of the allotransplant and the ethical conflict of using embryonic stem cells. The recent use of autologous or allogenic stem cells has been suggested as an alternative therapeutic approach for treatment of cartilage defects (Jung et al., 2009). MSCs have the capability to self-renew and are responsible for repair and repopulation of damaged tissues in the adult (Hombach-Klonisch et al., 2008). For these reasons MSCs are a promising cell resource for tissue engineering and cell-based therapies (Pittenger, 2008). The interest in MSCs and their possible application in cell therapy have resulted in a better understanding of the basic biology of these cells. Due to the low number of MSCs that can be isolated from a tissue sample, culture expansion is necessary to obtain adequate cell numbers for clinical purposes and for the analysis of molecular mechanisms. However, the number of mitotic divisions of MSCs in culture must be limited because MSCs age during *in vitro* culture, causing a reduction in their proliferative capacity (Banfi et al., 2000; Bonab et al., 2006) and gradual loss of the potential for multiple differentiation (Banfi et al., 2000; Izadpanah et al., 2006). The conservation of phenotype and differentiation capacity of MSCs are proportional to telomerization (Abdallah et al., 2005). Telomeres are normally shortened in successive cell divisions, however, in embryonic stem cells the telomere length is restored by telomerase enzyme activity. On the other hand, MSCs lack (Zimmermann et al., 2003) adequate levels of telomerase activity to achieve telomeric restoration (Izadpanah et al., 2006; Parsch et al., 2004; Yanada et al., 2006). Patient age also influences the characteristics of MSCs because their proliferative capacity is reduced by aging (Stenderup et al., 2003).

Three criteria define all types of stem cells: self-renewal, multipotency and the ability to reconstitute a tissue *in vivo*. According to a recent proposal of the International Society for Cellular Therapy (Dominici et al., 2006), MSCs are multipotent nonhematopoietic

progenitors located within the stroma of the bone marrow and other organs that are phenotypically characterized by the expression of several markers (e.g., CD73, CD90, and CD105) and the lack of expression of CD14 or CD11b, CD19 or CD79α, CD34, CD45 and HLA-DR surface molecules (Mrugala et al., 2009; Kastrinaki et al., 2008). Because there is no specific marker for MSCs, the principal criteria for identification are adherence to the plastic of the tissue culture flask, fibroblast-like morphology (Prockop, 1997), the prolonged capacity for proliferation in supportive media and the capacity to differentiate *in vitro* into cells of mesodermal origin (chondrocytes, adipocytes, osteoblasts). Furthermore, characteristics of MSCs are the absence of expression of typical hematopoietic antigens like CD34 and CD45, and the expression of surface markers like Stro-1, CD44, CD73, CD90, CD105 and CD166 (Pittenger et al., 1999).

Human MSCs, which are probably responsible for normal tissue renewal, as well as for response to injury (Tsai et al., 2007), have been isolated from several tissues, including bone marrow (Kastrinaki et al., 2008; Yoo et al., 1998), periosteum (Nakahara et al., 1990), perichondrium (Dounchis et al., 1997), synovial membrane (De Bari et al., 2001; Fickert et al., 2003), articular cartilage (Alsalameh et al., 2004); connective tissue of dermis and skeletal muscle (Young et al., 2001), peripheral blood (Villaron et al., 2004; Kuznetsov et al., 2001; Zvaifler et al., 2000), adipose tissue (Zuk et al., 2001 & 2002), lung (In´t Anker et al., 2003), liver (Le Blanc et al., 2005), amniotic fluid (You et al., 2008; Steigman & Fauza, 2007; Fauza, 2004), placenta (Barlow et al., 2008, Steigman & Fauza, 2007; Fauza, 2004: Matikainen & Laine, 2005), amniotic membrane (Díaz-Prado et al., 2010a & 2010b; Alviano et al., 2007), umbilical cord (Baksh et al., 2007) and umbilical cord blood (Mareschi et al., 2001). Although bone marrow is the usual source of MSCs, umbilical cord blood is emerging as an important reservoir for stem cells capable of differentiation into many cell types and possessing the advantages of immune status and relatively unshortened telomere length (McGuckin et al., 2005). Some countries have private and public stem cell banks from umbilical cord blood (UCB) for transplant programs or personal use (Samuel et al., 2008). Multipotent MSCs are a promising cell resource for tissue engineering and cell-based therapeutics because of their ability to self-renew and differentiate into specific functional cell types (Tsai et al., 2007). The list of tissues with the potential for tissue engineering is increasing because of recent progress in stem cell biology (Bianco & Robey, 2001).

In vitro (Pittenger et al., 1999; Majumdar et al., 1998; Muraglia et al., 2000) and in vivo (Gronthos et al., 2003) studies of clonally-derived MSCs demonstrated that the MSC population consists of subsets that have different expression of markers and different capacities for cellular differentiation. To improve the number of MSCs isolated from a tissue it is frequent to use a pre-plating technique that minimizes the number of contaminating fibroblasts in the culture (Richler & Yaffe, 1970). Also, MSCs show phenotypic and functional differences depending on their tissue of origin. For example, MSCs from bone marrow and synovial membrane have been differentiated by their gene expression profiles (Djouad et al., 2005).

Several studies have recently reported the migration of intraarticularly injected MSCs to the site of a cartilage injury to repair chondral defects. In a caprine model for osteoarthritis in which OA is induced by the complete excision of the medial meniscus and resection of the anterior cruciate ligament, the intraarticular injection of MSCs produced meniscus repair after 6 weeks; however, there was no evidence of cartilage or ligament repair (Murphy et al., 2003). This suggests that the injected MSCs migrated to the injured meniscus, but not the

damaged cartilage. The intraarticular injection of MSCs into rat knees, however, showed mobilization of these cells towards all injured tissues, including articular cartilage; the MSCs contributed to tissue regeneration (Nishimori et al., 2006; Agung et al., 2006).
In osteoarthritic knees, MSCs embedded in collagen gel were implanted into chondral defects and closed with periosteal membrane. After 42 weeks, arthroscopic and histological results were better than in osteoarthritic patients without implants, although there was no statistically significant improvement in clinical results (Wakitani et al., 2002). The use of MSCs to treat chondral lesions clinically has not been established, in part because the stages of chondrogenic differentiation of MSCs are not sufficiently defined. In addition, there are currently no protocols that ensure direct differentiation to the desired phenotype; the plasticity of the cells differentiated from MSCs can lead to undesirable phenotypic alterations (De Bari et al., 2004; Pelttari et al., 2006).

10. Scaffolds

The clinical outcome of the techniques described above underline the need of increase the quality of the synthesized repair tissue. To overcome some of the limitations of ACI, cell delivery supports can be used for cell transplantation. Recent research efforts have focused on tissue engineering as a promising approach for cartilage regeneration and repair (Kuo et al., 2006). Tissue engineering is a technique by which a living tissue can be reconstructed by associating the cells with biomaterials that provide a scaffold on which they can proliferate three-dimensionally, under physiological conditions (Iwasa et al., 2009). A biomaterial is any pharmacologically inert compound designed to be implanted or incorporated into the living system. Therefore cartilage tissue engineering is critically dependent on the selection of appropriate cells (differentiated or MSCs), suitable scaffolds for cell delivery and biological stimulation with chondrogenically bioactive molecules (Kuo et al., 2006). The transplantation of chondrocytes seeded on natural and synthetic scaffolds has been used for cartilage tissue engineering (Kuo et al., 2006). Regeneration of a hyaline-like repair tissue could be obtained after the implantation of a pre-engineering, functional cartilage tissue, instead of the delivery of a chondrocyte implantation (Pelttari et al., 2009). A major prerequisite for choosing a scaffold is the property of not producing toxic, injurious, carcinogenic, or immunological responses (either inflammation or rejection) in living tissue (Niknejad et al., 2008). New tissue regeneration should occur as the scaffold degrades, so the new tissue assumes the shape and size of the original scaffold. Design criteria for scaffolds include suitable mechanical strength and surface chemistry, ability to be processed in different shapes and sizes, and the ability to regulate cellular activities such as differentiation and proliferation (Kuo et al., 2006). Moreover, requirements for the biomaterials used as a scaffold include controlled biocompatibility, structurally and mechanically stable, permeability (allowing the exchange of nutrients and metabolites), suitable ligands for implanted cell attachment, must support the loading of an appropriate cell source to allow successful infiltration and attachment with appropriate bioactive molecules in order to promote cellular differentiation and maturation. Also, they must present readily integration with native cartilage, biodegradation into non-toxic products that can be replaced by host cells, initial stability and provide an excellent environment for cell and tissue growth and differentiation crucial to maintain cell function and development of new tissue. Scaffolds must also provide a stable temporary structure while cells seeded

within the biodegradable matrix synthesize a new and natural tissue (Frenkel & Di Cesare, 2004). Other important factors in the design of a scaffold are pore size, porosity, adaptive shape, mechanical integrity, the ability to be retained at the implantation site and cost efficiency.

A number of scaffolds have been developed and investigated, in vitro and in vivo, for potential use in tissue engineering and in particular for in vitro regeneration of cartilage tissues (Vinatier et al., 2009). Carries have been marketed and various tissue-engineering techniques have been developed using chondrocytes seeded on biological matrices (Iwasa et al., 2009). For cartilage tissue engineering, scaffolding has been fabricated from both natural and synthetic polymers (Tuli et al., 2003), such as fibrous structures, porous sponges, woven or non-woven meshes and hydrogels (Kuo et al., 2006). Natural biomaterials, such as fibrin, collagen, agarose, alginate, hyaluronic acid or chitosan (Eyrich et al., 2007; Cao & Xu, 2008; Mouw et al., 2005; Lisignoli et al., 2006; Nettles et al., 2002) and synthetic biomaterials, such as poly-lactic glycolic acid (PLGA) (Han et al., 2008) and a polymeric nanofiber (Janjanin et al., 2008), are used alone or in different combinations to make scaffolds. Collagen and hyaluronan-based matrices are among the most popular natural scaffolds in clinical use nowadays, since they contain natural components of the hyaline cartilage. On the contrary, there is no clinical experience using scaffolds such as alginate, agarose and chitosan (Iwasa et al., 2009). Within each kind of biomaterial (natural and synthetic) there are many types of biomaterials that are being studied, with controversial results. The human amniotic membrane (HAM) is considered to be an important potential source for scaffolding material (Niknejad et al., 2008). The HAM possesses clinical considerable advantages that are not shared by other natural or synthetic polymers. On the other hand, HAM has abundant natural cartilage components, which are important in the regulation and maintenance of normal chondrocyte metabolism (Jin et al., 2007); this suggests that the HAM is an excellent candidate for use as native scaffold for cartilage tissue engineering (Niknejad et al., 2008). Amnion allografts are widely applied in ophthalmology, plastic surgery, dermatology, and gynecology (Tejwani et al., 2007; Santos et al., 2005; Rinastiti et al., 2006; Meller et al., 2000; Morton & Dewhurst, 1986). A recent study demonstrated the potential use of the HAM as a scaffold to support human chondrocyte proliferation in cell therapy to repair human OA cartilage (Díaz-Prado et al., 2010c).

Experimental studies in animals with synthetic biomaterials showed disappointing results, since after 8 weeks of implantation, all animals suffered ulceration and loss of cartilage (Oka et al., 1997). The problem that arises with artificial biomaterials is that the implant is not interwoven with adjacent bone, leading to degradation of the recovered surface after only 2 or 3 months (Oka et al., 1997). In a study in rabbits with a biomaterial composed of collagen in which chondrocytes were seeded, a good proliferation and cell phenotype maintenance were shown; therefore good repair results were observed (Frenkel et., 1997). One of the major limitations of the use of matrices is the size of the lesion (Nixon et al., 1993, Sams & Nixon, 1995, Sams et al., 1995). Despite the diffusion of new tissue-engineering techniques and the number of scaffolds that have been investigated, the ideal matrix material has not been identified. However, the clinical use of these materials is currently limited, mainly due to the risk of disease transmission and immunoreaction (Iwasa et al., 2009).

Mechanical and biological properties of biomaterials significantly influence chondrogenesis and the long-term maintenance of the structural integrity of the neo-formed tissue. The three-dimensional nature of the scaffolds promotes maintenance of rounded cell

morphology and the elevated expression of glycosaminoglycans and type II collagen (Nettles et al., 2002; Gong et al., 2008). Other advantage is that cell delivery supports may act as barrier to the invasion of the graft by fibroblasts, which may otherwise induce fibrous repair (Frenkel et al., 1997). Indeed, the presence of ECM around cells was reported to increase donor cell retention at the repair site and possibly protect the cells from environmental factors such as inflammatory molecules (Pelttari et al., 2009). The tissue-engineering methods with scaffolds including the arthroscopy technique are less invasive because there is no need to harvest periosteum (Iwasa et al., 2009). Other benefits of this methodology are: reduce surgical time, morbidity, and risk of periosteal hypertrophy and postsurgical adhesions substantially (Iwasa et al., 2009). However, scaffolding biomaterials have differing influences on the metabolism of host cells and, consequently, the quality of the tissue-engineered cartilage (Mouw et al., 2005, Jeon et al., 2007). For example, the use of chitosan, compared to PLGA, for cartilage tissue engineering produces a superior maintenance of structural integrity because the expression of type II collagen protein and mRNA became weaker over time in the PLGA group (Jeon et al., 2007). Scaffolds using hyaluronic acid are also being used with excellent clinical and histological results (Giannini et al., 2008).

11. Gene therapy

The introduction of genetic products into the field of tissue damage repair can enhance the process of articular cartilage restoration. The most obvious would be growth factors, proteinase inhibitors and cytokine antagonists. The gene therapy process involves the determination of the appropriate gene and cell type (chondrocytes, chondrogenic cells and cells of the synovial membrane) for the gene transfer, as well as the determination of the optimal vector to incorporate the cDNA (Trippel et al., 2004). Different anabolic factors, such as members of the TGF-β3 (tumor growth factor beta 3), IGF (insulin growth factor), FGF (fibroblastic growth factor), and HGF (hepatocyte growth factor) superfamily, could induce chondrogenesis and the synthesis of ECM components, while anti-inflammatory molecules, such as interleukins (IL): IL-4, IL-10, Il-1Ra (IL-1 receptor antagonist), and TNFsR (tumor necrosis factor soluble receptor), could act as inhibitors of cartilage degradation (Gelse et al., 2003).

The synovial membrane seems to be useful as a target for chondroprotective therapies (Palmer et al., 2002). The viral transfection *in vivo* with the IL-1Ra gene in rheumatoid arthritis joints reduces the severity of the disease process in animal models (Gouze et al., 2003). Furthermore, this technique makes possible the safe intraarticular expression of the IL-1Ra gene (Evans et al., 2005 & 2001). Chondrocytes and MSCs are the preferred targets for the induction of chondrogenesis. Using animal models, the transplantation *in vivo* of MSCs transfected with BMP-2 (bone morphogenetic protein-2) cDNA produces improved chondral lesion repair with a higher production of proteoglycans and type II collagen compared to controls (Park et al., 2006).

12. Conclusion

Modalities of cellular therapy to repair focal articular cartilage defects include the implantation of cells with chondrogenic capacity and creating access to the bone-marrow. Of the numerous treatments available nowadays, no technique has yet been able to consistently

regenerate normal hyaline cartilage. The implantation of autologous chondrocytes and autologous mosaicplasty induces a better quality of articular cartilage whereas the use of stem cell implants is in an early experimental stage at this time. Currently the autologous chondrocyte implantation is the most effective therapeutic alternative to repair focal articular cartilage lesions although this procedure is also used for patients with osteochondritis dissecans but not for osteoarthritis joints. On the other hand the use of tissue-engineered grafts based on scaffolds seems to be as effective as conventional ACI clinically but there are no convincing evidences that scaffold techniques allow the maintenance of the chondrocyte phenotype and the homogeneous distribution of the cells. Therefore it has not verified that the technical and theoretical advantages of scaffold techniques have led to the better clinical and histological results compared with conventional ACI. Further studies would be needed to determine whether articular cartilage repair with scaffolds is the most adequate alternative to ACI.

13. Acknowledgements

This study was supported by grants: Servizo Galego de Saúde, Xunta de Galicia (PS07/84); Cátedra Bioiberica de la Universidade da Coruña; Instituto de Salud Carlos III CIBER BBN CB06-01-0040; Ministerio de Ciencia e Innovacion PLE2009-0144; Fondo de Investigacion Sanitaria-PI 08/2028 with participation of fundus from FEDER (European Community), Silvia Diaz-Prado is beneficiary of an Isidro Parga Pondal contract from Xunta de Galicia, A Coruna, Spain.

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Biomedical Engineering, Trends, Research and Technologies

Edited by Dr. Sylwia Olsztynska

ISBN 978-953-307-514-3
Hard cover, 644 pages
Publisher InTech
Published online 08, January, 2011
Published in print edition January, 2011

This book is addressed to scientists and professionals working in the wide area of biomedical engineering, from biochemistry and pharmacy to medicine and clinical engineering. The panorama of problems presented in this volume may be of special interest for young scientists, looking for innovative technologies and new trends in biomedical engineering.

How to reference

In order to correctly reference this scholarly work, feel free to copy and paste the following:

Silvia M Díaz Prado, Isaac Fuentes Boquete and Francisco J Blanco (2011). Cell Therapy and Tissular Engeenering to Regenerate Articular Cartilage, Biomedical Engineering, Trends, Research and Technologies, Dr. Sylwia Olsztynska (Ed.), ISBN: 978-953-307-514-3, InTech, Available from: http://www.intechopen.com/books/biomedical-engineering-trends-research-and-technologies/cell-therapy-and-tissular-engeenering-to-regenerate-articular-cartilage

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