Chapter 21

The Use of Mesenchymal Stem Cells in Orthopedics

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Abstract The use of mesenchymal stem cells in orthopedics has been postulated for many years, with robust animal data showing efficacy in cartilage healing, tendon repair, and intervertebral disc treatment. Early clinical data is also just starting to be published in primarily cartilage repair and these results are encouraging. The field of tissue engineering with stem cells in orthopedics has the potential to reduce morbidity and improve clinical outcomes.

Keywords Mesenchymal stem cells · Stem cells · Orthopedics · Autologous cells · Bone marrow · Adipose tissue · Differentiation

Introduction

Mesenchymal stem cells (MSCs) are multipotent, adult stem cells that show great clinical potential as therapeutic agents in regenerative medicine (Barry, 2003). They are also known as marrow stromal cells and are derived from the mesoderm. Scientists Ernest McCulloch and James Till first discovered the clonal nature of a sub-population of marrow nucleated cells in the 1960s (Becker et al., 1963). Friedenstein elucidated that these stromal cells were capable of forming colonies and could be assayed in this way and coined the term "Colony Forming Units" (CFUs) (Friedenstein et al. 1974). Experiments through the 1980s and 1990s demonstrated that environmental clues helped MSCs differentiate into different cell types. For example, culturing with ascorbic acid, inorganic phosphate, or dexamethasone could differentiate cells to osteoblasts, while culturing in the presence of TGF-beta caused cells to differentiate into chondrocytes (Alhadlaq and Mao, 2004).

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Cell Sources

MSCs can be easily isolated from many anatomic locations, including marrow aspirate, marrow mobilized blood, muscle, adipose, and other tissues (Alhadlaq and Mao, 2004). For orthopedic purposes, these sources have been compared by many authors for their ability to heal bone and cartilage and there are measureable differences in this regard. As a rule, the closer the source is to the target tissue being repaired, the more effective the MSCs appear to be at differentiation to the target tissue type. For example, Vidal compared equine MSCs derived from bone marrow (bm-MSCs) vs. adipose tissue (a-MSCs) for chondrogenic potential and found that bm-MSCs produced a more hyaline like matrix and had better glycosaminoglycan production (Vidal et al., 2008). Keeping with this trend, Yoshimura determined that synovial derived MSCs (closest to the target tissue of cartilage) had better chondrogenesis than bm-MSCs (Yoshimura et al., 2007). From a pragmatic standpoint, in our day to day experience, obtaining enough synovium to produce a critical amount of MSCs may be difficult as it is a more limited resource than bone marrow aspirate. Once way to sidestep this issue may be to obtain synovial derived MSCs from synovial fluid (SF), a more renewable and

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easier obtained source. Our lab's experience with this tissue as a source has shown significant variability in the ability to obtain SF from osteoarthritic knees. In addition, the ability to culture cells from fluid has also been variable.

Stem Cell Culture

A limited amount of cells can be obtained from any tissue. In many instances, the amount that can be obtained from tissue is less than the critical quantity of cells that are needed for tissue repair. One method of obtaining more cells is culture expansion, or growing cells in culture to larger numbers. MSCs are usually culture expanded via monolayer culture, which is a process that involves seeding a certain density of cells onto a specialized flask, where the cells attach to a plastic surface and begin to form colonies. The cells are fed through a nutrient broth that is maintained above the plastic surface. Because MSCs are contact inhibited, they will grow in culture until they become confluent and then abruptly stop propagating. To keep cells proliferating in culture, when the colonies are near confluence, the non-adherent cells are discarded and an enzyme like trypsin is used to detach the MSCs from the plastic surface. The MSCs are then re-plated in a similar flask and the media changed with this process being known as a "passage". Most MSCs in culture are grown to the 2nd–5th passage, as some studies have shown decreased differentiation if MSCs are grown for prolonged periods in culture with a higher chance of genetic mutation (see Fig. 21.1) (Banfi et al., 2000). Note that the percentage of adherent cells vs. non-adherent cells increases with each passage and most studies consider that a "pure" MSC population is obtained after approximately the second passage.

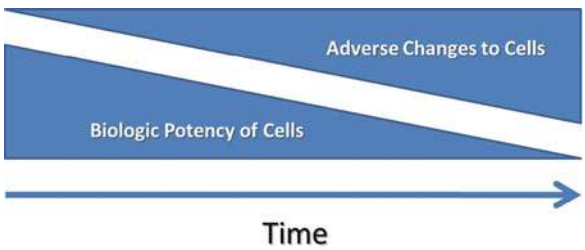

Fig. 21.1 Adverse changes in cells increase with time in culture as biologic potency decreases

Differentiation vs. Paracrine Effects

Animal studies have demonstrated the multipotency of MSCs, and how they can differentiate into muscle, bone, cartilage, tendon, and various cells of internal organs as well as exhibit paracrine effects to assist in tissue repair. In this context, paracrine means that MSCs release certain growth factors to assist in tissue repair (Ladage et al., 2007). These include TGF-beta, VEGF, FGF, and other signaling factors that can help recruit other cells to the local area. Many authors have questioned whether most of the positive repair effects observed in experimental MSC therapies are due more to this paracrine signaling than differentiation of cells.

Autologous vs. Allogeneic

Autologous stem cells obviously do not have the same communicable disease transmission risk as allogeneic cells. While for most patients, autologous stem cell therapy may be suitable, some studies have shown a lower differentiation potential in older patients (Zhou et al., 2008). As a result, some have postulated that allogeneic cells may be better suited for these patients. In addition, allogeneic cells should be able to be mass produced in bioreactors, proving a ready supply of cells for therapy. However, some concerns have been raised about the use of allogeneic stem cells. As an example, Ueda et al. (2007) recently found that stem cells transplanted from the bone marrow of mice bred to have osteoporosis were able to induce osteoporosis in normal healthy mice, indicating that stem cells may be a genetic disease vector. In addition, many have argued that allogeneic MSCs are immune-privileged as they lack major histocompatibility complexes; however, Prigozhina et al. (2008) have found that allogeneic MSCs lose their immunosuppressive potential in a mismatched setting.

Animal Data: Cartilage Repair

Some of the earliest models of cartilage repair used autologous, cultured chondrocytes. However, the complications of using chondrocytes for cartilage repair included hypertrophy, graft failure, long culture times, and the invasiveness of the implant

procedure (Nejadnik et al., 2010). Because MSCs are multi-potent, animal models of cartilage repair using MSCs started to appear in the literature in the early 1990s (Caplan, 1999). In many of these studies, an osteochondral defect (OCD) was created experimentally and the MSCs were implanted into the lesion, usually in a hydrogel or other carrier. Partial to robust healing of the defect took place over weeks to months (Alhadlaq and Mao, 2004).

MSCs are delivered to OCDs in many different biologic scaffolds including hydrogels, fibrin, in native extra-cellular matrix, collagen, or in a suspension. A scaffold is a matrix with properties that support cell migration, attachment, three dimensional position, and engraftment. Based on unpublished data, we have noted that stiffer biologic scaffolds (like dense fibrin glue) tend to reduce MSC viability as they limit MSC movement through the material. On the other end of the spectrum is using no scaffolding, or delivering cells in suspension and allowing them to attach to the repair site. The research into this technique is interesting as it highlights how MSCs work-through attachment. For example, Fig. 21.2 shows histology adapted from Koga et al. (2008) demonstrating minimal cartilage repair with a control saline injection, minimal repair with MSCs injected intra-articular, and robust repair when MSCs were allowed to attach to the lesion via gravity. As a result, we would hypothesize that exact placement of MSCs in a joint is very important.

Surgical techniques for cartilage repair have long relied on bone marrow derived stem cells. For example, the micro fracture procedure relies on creating holes in the osteochondral plate and allowing whole marrow to clot in the osteochondral defect. While this technique has had some success in younger, athletic patients, the cartilage produced by this low concentration of MSCs in the clot tends more toward non-native fibrous cartilage versus the more hyaline like cartilage produced by higher concentrations of cultured MSCs (Mobasheri et al., 2009). Recently, a proof of concept study in an equine model was published by McIlwraith et al. (2010), showing better repair with a combination of micro fracture plus MSCs than microfracture alone. The repaired tissue was significantly firmer and had higher levels of aggrecan, a molecule that provides compressive stiffness to cartilage.

Animal Data: Meniscus Repair

The challenge in repairing the meniscus has been due largely to the poor blood supply of the inner 2/3'rds (white zone) of the structure versus the excellent blood supply of the outer 1/3 (red zone). Interestingly, Izuta et al. demonstrated that cultured MSCs may be able to overcome this problem of poor repair in the avascular zone. His group was able to demonstrate meniscus repair in the white zone when MSCs were transplanted into this area using a fibrin matrix (Izuta et al., 2005). Of note, Agung et al. (2006) reported a murine model of intra-articular injection after acute injury of multiple knee structures, including the meniscus. This model demonstrated that for blind intra-articular injection (rather than the local adherent model proposed by

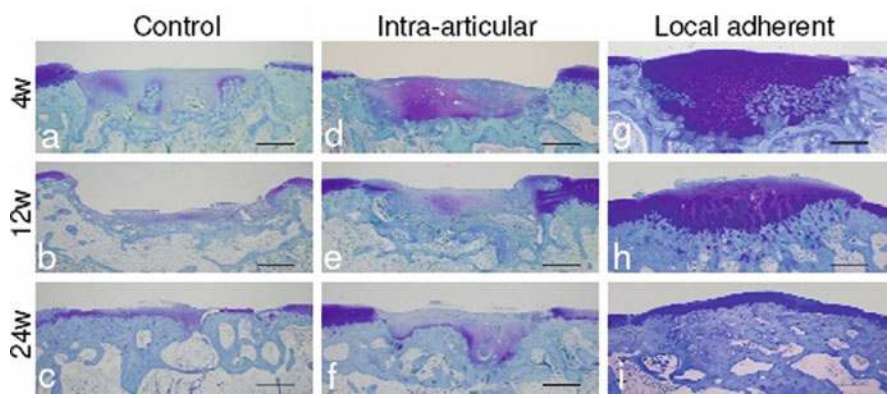

Fig. 21.2 The healing of experimental osteochondral defects (OCDs) using different methods of applying MSCs. The control OCD was injected with saline and shows poor healing at 24 weeks. The "Intra-articular" defect was treated with MSCs that were blindly injected into the joint and demonstrated very little healing. Finally the "Local Adherent" lesion was treated by dripping MSCs directly on the lesion and shows robust healing (adapted from Koga et al., 2008)

Koga), the number of cells injected was related to their ability to be found in the meniscus. For example, at a dose of 1×10^6 MSCs, none were found in the injured meniscus but at a dose of 1×10^7 cells, MSCs were generally found in this area. This may fit with Koga's hypothesis, as a higher number of cells injected into the joint would make it more likely that cells would be able to attach at the site in need of repair. Horie et al. (2009) reported that synovial derived MSCs that were injected into massive rat meniscus tears were able to differentiate and repair meniscal tissue. Interestingly, the authors also demonstrated that these cells did not migrate out of the knee to distant organs.

Animal Data: Tendon Repair

Tendons are often difficult to repair without a high level of morbidity or re-rupture. Awad has published a rabbit model showing that cultured MSCs were able to speed tendon healing and produced better tendon appearance than non-MSC treated tendons. Importantly he noted better maximum stress, modulus, and strain energy density as well as minor improvements in the histological appearance of some of the MSC-mediated repairs, including increased number of tenocytes and larger and more mature-looking collagen fiber bundles (Awad et al., 1999). However, he was unable to show better morphometrics when compared to the non-MSC treated sides. Chong et al. (2007) also demonstrated improved modulus in resected rabbit Achilles tendons treated with MSCs and morphometric changes, concluding that MSCs can improve the histological and biomechanical parameters in the early stages of tendon-healing. Both of these studies are in contrast to Gulotta et al. (2009), whose animal model of surgical rotator cuff tendon healing showed no differences between MSC treated and untreated groups.

Animal Data: Intervertebral Disc

Perhaps no area captures the imaginations of physicians like repairing intervertebral discs with stem cells. This is perhaps because traditional surgical approaches continue to show disappointing results (Fritzell et al., 2003). Animal models of disc repair using MSCs are abundant, with successful murine, canine, rabbit, and ovine models. Sakai et al. (2003) have published several papers on the topic whereby MSCs are usually combined with atellocollagen and inserted into an experimentally created degenerative disc in a rabbit model. They have observed improvements in MRI disc hydration, height, and morphology. Risbud et al. (2004) have investigated the coculturing of MSCs with cells from the nucleus pulposis (NP) showing that this technique can produce partially differentiated cells that are capable of repopulating the NP. Risbud et al. (2004) has used different methods for MSC differentiation toward the NP phonotype including using hypoxia and TGF-beta in culture. Zhang et al. (2005) has shown that MSCs injected into discs without pre-conditioning or coculture can help to increase proteoglycan production in the NP. Finally, Miyamoto et al. (2010) recently demonstrated that intra-discal transplantation of synovial derived MSCs prevented disc degeneration through suppression of catabolic genes. In summary, while the results from animal models are impressive, questions remain as to whether a quadruped disc with its very different load characteristics can serve as an adequate model for biped disc repair. In addition, in all of the animal models studied to date, an artificially created degenerated disc (acute disc stab model) is used as a surrogate for the chronic degenerated discs normally encountered in patients (Yoshikawa et al., 2010).

Model for Cell Delivery in Orthopedics

Delivery of cells into a joint to treat orthopedic injuries could take two common routes used daily in clinical practice: percutaneous injections and arthroscopic placement. Injecting cells into a confined space such as infiltrating into soft-tissues will likely result in the MSCs that stay local to the injection site. However, as discussed above, injecting in a large joint presents some concerns, as multiple animal models have shown that cells may or may not find their way into the damaged areas (Agung et al., 2006). Because MSCs function through local attachment to the damaged site, data presented by Koga et al. (2008) showing that MSCs dripped on a lesion produce better repair is encouraging as a model for injection (i.e., slow injection onto a lesion). Finally, since MSCs are capable of chemotaxis, placing certain growth factors on the injured tissue may result in more MSCs accumulating at the

target site (Fiedler et al., 2002). Another challenge in MSCs delivery is that most arthroscopic surgery is performed in an aqueous medium. For cells in suspension, this presents a challenge, as the MSCs would easily be whisked away from a surgical re-implant site by the action of arthroscopy pumps designed to clean debris from the operative field. As an alternative, Nejadnik et al. (2010) have used a surgical approach similar to autologous chondrocyte implantation, where MSCs are placed in a dense hydrogel and sutured under a protective membrane. Another alternative method is to culture the MSCs into a tissue engineered construct (TEC), allowing the cells to produce their own extracellular matrix. This TEC approach produces a pea sized collection of cells that are kept in an undifferentiated state for prolonged periods and can be placed under water arthroscopy in their own natural biologic scaffold (Ando et al., 2007).

Clinical Studies in Orthopedic Diseases

We have previously described several case studies in which positive MRI changes were observed in knees treated with culture expanded MSCs, corresponding with symptomatic improvement (Centeno, Busse et al. 2008). We have also reported on the complication rate of human culture expanded MSCs used for orthopedic purposes, noting a rate no greater than with other needle-based interventional techniques directed at peripheral joints (Centeno et al., 2010). In submitted, but yet unpublished data on 339 patients, this safety profile was continued at up to 4 years post MSC reimplantation. Other authors have described similar results using more invasive surgical implant techniques: Wakatani has reported on a 11-year prospective study of 45 knees (in 41 patients) treated with autologous bone marrow-derived MSCs, with results indicating both safety and efficacy (Wakitani et al., 2010). Nejadnik et al. (2010) and colleagues recently described a comparison between surgically implanted chondrocytes versus MSCs harvested by needle in 72 knees of older patients. They demonstrated good safety, less donor site morbidity, and better efficacy for the MSC treatment when compared with an autologous chondrocyte procedure. Haleem et al. (2010) noted that autologous, cultured bm-MSCs re-implanted into articular cartilage defects in platelet rich fibrin demonstrated evidence of healed cartilage in most patients at 12 months post-operative. While very little has been published on intervertebral disc repair in humans, some clinical data is available. Yoshikawa et al. (2010) recently published on two patients who were treated with surgically implanted MSCs that were cultured using a serum free technique. After 2 years, no complications were noted and both patients showed improvements in vacuum phenomena on follow-up MRI. The only other human data of which we are aware is that produced by our group from 2005 to 2010 under IRB supervision and now being readied for publication. Our experience demonstrated that placing a bone marrow nucleated cell fraction (an enriched MSC population with other cells) into the disc via percutaneous means produced no measureable clinical or MRI results in patients with degenerative disc disease. The next series involved replication of the Sakai study (Sakai et al., 2003), where cultured MSCs were placed into the disc in a similar patient population and again this technique produced measureable results. Finally, a third case series was performed where changes were made in culture and injection technique as well as the diagnosis being treated (changed from DDD to chronic disc bulge causing lumbar radiculopathy). This last model showed encouraging clinical and imaging results.

Implications in Real World Clinical Applications

To consider the real world implications of viable cell based alternatives to more invasive orthopedic surgeries, total knee arthroplasty (TKA) is an interesting model. Knee replacement surgery, also called knee arthroplasty, has been employed increasingly over the past 10 years as a means of treating symptomatic degenerative changes of the knee. It is estimated from discharge data from the Nationwide Inpatient Sample (NIS) of the Healthcare Cost and Utilization Project (HCUP), that the number of partial and total knee replacement procedures among U.S. patients 65 years and older increased from 178,653 in 2000 to 357,472 in 2008, a 100% increase (HCUP, 2008). In contrast, we have recently submitted for publication a large case series of 250 knee and hip osteoarthritis patients treated with percutaneous injection of MSCs. As an

example, while 2/3'rds of the knee patients were TKA candidates, only 6% of the patients opted for TKA despite the injection. In addition, statistically significant differences in reported outcome between the treatment and an untreated control were observed. Finally, complications rates were minimal compared to TKA.

Regulatory Processes

The regulatory environment in the United States and Europe for stem cells that are more than minimally manipulated has largely considered these cells the same as mass produced drugs. This category includes cultured cells, using any cell for a non-homologous use (for example an adipose MSC for an orthopedic indication), and any significant processing of cells beyond a simple centrifugation. This means that prolonged approval processes are often used to bring these technologies from the bench to the bedside. While this approach may certainly make sense for mass produced cells being distributed en masse in vials, this same approach is also applied to autologous cells. As a result, real world clinical knowledge on the use of stem cells in patients is largely accumulating outside the U.S. and Europe.

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