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Research Article

Mesenchymal Stem Cell Therapy in Cartilage Injuries of Knee – Review

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Abstract

Full-thickness cartilage injuries when left alone would increase the risk of osteoarthritis (OA) with severe associated pain and functional disability. The capability of the mesenchymal stem cells (MSCs) to repair and regenerate cartilage has been widely investigated. Articular cartilage defect may result from direct trauma or chronic degeneration. The subchondral bone plays an important role in healing through the presence of mesenchymal elements.

MSCs are the most representative adult stem cells. It was observed that BM-MSCs (Bone Marrow derived MSCs) had superior chondrogenic differentiation capacity as compared to MSCs from other origins. It has been observed that a MSC density of 5×10^7 or 5×10^8 cells per milliliter embedded in a collagen gel had more proteoglycans than lower cell densities, better facilitating cartilage defect healing. The most potent chondrogenic differentiation inducers are transforming growth factor β , bone morphogenic protein, fibroblast growth factor, and insulin-like growth factor 1.

Most commonly used delivery system for MSCs are either sealant or scaffold based. Ideal scaffold should have similar characteristics to the native tissue. The scaffold has the advantage of withstanding the in-vivo loading environment and protects the embedded cells. There is contradictory evidence regarding injectable treatment with BM-MSCs suggesting scaffolds may be required for the regeneration of cartilage. There is high quality of evidence to support MSC therapy but further refinement of methodology will be required to support its routine clinical use. There are few options to be explored e.g. MSC exosomes, genetic engineering and epigenetic regulatory mechanisms governing MSC biology.

Introduction

Sporting population is prone to multitude of injuries around the knee joint. Cartilage of each human joint is a highly specialised tissue acting as a shock absorber, enabling synovial joints to articulate with low frictional forces. It has limited repair potential since it is not vascular, nor it has lymphatics and largely aneural [1]. Full-thickness cartilage defects if left alone would increase the risk of osteoarthritis (OA) with severe associated pain and functional disability. The current methods for articular cartilage (AC) repair include non-surgical and surgical alternatives. A wide range of surgical approaches is being practiced. The spectrum includes use of arthroscopy with micro fracture and micro drilling, soft tissue grafting, osteochondral transplantation, and autologous chondrocyte implantation (ACI) [2-4]. Brittberg in 1994, first transplanted patients own chondrocytes into the articular defect site of a knee [5]. AC defect may result from one of the following mechanisms:

- a) Trauma (direct or indirect);
- b) mechanical overloaded (chronic degeneration); and
- c) Subchondral bone changes (osteochondritis dissecans, avascular necrosis).

The potential for spontaneous regeneration of the cartilage is low. The cartilage breakdown occurs due to production of redundant proteolytic enzymes. The inflamed synovium produces catabolic and proinflammatory factors (prostaglandin E₂, Nitric Oxide) which alter the equilibrium of cartilage matrix metabolism [6]. The subchondral bone plays an important role in healing of profound defects through the presence of mesenchymal elements. These elements proliferate to form a connective tissue of fibrous nature, which gradually differentiates into a lower-quality fibrocartilage.

Successful tissue engineering in articular cartilage repair has four components:

- i. Specific cell types, which can proliferate, differentiate and maintain the phenotypic properties.
- ii. A scaffold to provide an adequate 3-dimensional environment for the cells to grow,
- iii. Addition of appropriate chemical factors such as growth factors, cytokines or hormones as a suitable stimulus for specific lineage differentiation of the cells,
- iv. Cells require a microenvironment (physical and biochemical factors to regulate MSC behavior) which withstands the mechanical and biochemical state of the joints [7].

Role of Mesenchymal Stem Cells (MSCs)

Stem cells are classified into three main categories: embryonic stem cells (ESCs), induced pluripotent stem cells (iPSCs), and adult or somatic stem cells [8]. Adult stem cells may be derived from bone marrow, adipose tissue (fat), muscle, extra placenta, placenta, umbilical cord, synovial membrane, tendons and cartilage.

MSCs are the most representative adult stem cells and can be induced to differentiate into different mesenchymal lineages such as bone, cartilage, fat, ligament, tendon, and other connective tissues. Stem cells secrete mediators that promote endogenous growth, stimulate self-proliferation of progenitor cells, and inhibit chondrocyte apoptosis or cartilage degeneration, promote angiogenesis and decrease oxidative stress through regulating TGF β , VEGF, ADAMTS, MMPs, TIMPs achieving cartilage regeneration and cartilage protection [9].

The majority of the studies used bone marrow-derived MSCs (63%) followed by adipose tissue (33%) [10].

BMSCs may allow better differentiation of the deep calcified articular cartilage zone adjacent to healthy bone [11]. Thirty-



one published studies by 15 different groups looked at clinical applications of MSCs. One used allogenic stem cells and the rest autologous stem cells [10]. In vivo studies show that the regenerated cartilage is similar to fibrocartilage with inferior mechanical properties, healing capacity and requires sufficient cell numbers with chondrogenic potential of cells [12]. These limitations could be circumvented by use of iPSCs [13]. Using a defined quality control process and a chondrogenic differentiation protocol, hiPSCs (human induced pluripotent stem cell) may be the ideal cell source for cartilage tissue engineering [14].

Enhancing Chondrogenesis

MSC harvest, isolation & scaling up

In studies using bone marrow concentrate, approximately 60 ml of bone marrow aspirate was harvested and concentrated down to a volume of 2–4 ml before use [15–18]. Density gradient centrifugation is used to obtain Mononuclear (MNC) fraction of cells from bone marrow aspirate. For achieving high clinical efficacy, the estimated dosage of MSCs required is approximately 40 to 100 × 10⁶ cells per patient [17]. Therefore, ex-vivo cell expansion is a key step in the development process [19–21].

Cell seeding

Healthy AC naturally contains 9.6 × 10⁶ chondrocytes/cm [22]. It has been reported in clinical studies that scaffolds seeded with 5 × 10⁶ cells per scaffold induced the highest chondrogenesis [22,23] Yokoyama et al reported that a MSC density of 5 × 10⁷ or 5 × 10⁸ cells per millilitre embedded in a collagen gel had more proteoglycans than lower cell densities, better facilitating chondral defect healing and supporting the need to identify a MSC source with a high proliferation potential [24].

Growth factors

The most potent chondrogenic differentiation inducers are transforming growth factor β, bone morphogenetic protein, fibroblast growth factor, and insulin-like growth factor 1 [25–27]. The induction process can be enhanced by steroids.

Stem Cell Implantation

Sealant based

Most common sealant gels are collagen and fibrin having gluing properties and are biodegradable. Sealants may be natural or synthetic. Natural ones include collagen, gelatin, alginate, chitosan, chondroitin sulfate, agarose, hyaluronic acid (HA) and silk [28]. They are biodegradable, biocompatible and reduced immune response. Synthetic ones are polyethylene glycol (PEG), polyurethane (PU), and polyester which are biocompatible, strong adhesive and biodegradable.

Scaffold based

A scaffold is commonly used to facilitate in vitro chondrogenesis for tissue engineering. Ideal scaffold have been suggested as having similar characteristics to the native tissue, being a source of cells that could promote tissue regeneration, highly porous to permit cells penetration and tissue impregnation, high permeability for allowing delivery of nutrients and gas exchange, biocompatible and biodegradable once the functional tissue has been formed. Biodegradable polymers can be natural or synthetic. Natural biodegradable polymers are polynucleotides, polysaccharides and proteins, whereas synthetic biodegradable polymers include poly-lactic acid (PLA), poly-glycolic acid (PGA), and poly-lactic co-glycolic acid (PLGA) [29]. A systematic review observed 53 in vitro studies, natural scaffold (26) and synthetic scaffolds (9) and hybrids (18). The authors found the most popular being a fibrin-polyurethane scaffold [10].

Scaffold free 3D culture

Before the emergence of scaffolds, scaffold-free 3D culture systems were generally used for chondrogenesis. The popular methods used for cell-based therapy are pellet culture and micromass culture. The distribution, density, and matrix composition of cells in pellet culture are similar to native AC [30]. High-density micro mass culture induces cell-cell interaction, aggregating into a high density pre-cartilaginous core [31].

Intra-Articular Delivery of MSCs (Clinical Trials)

The method used to deliver MSCs to an articular defect is highly technical. The objective of surgical implantation is to create a 3-D environment that optimizes cell proliferation and differentiation.

Intra-articular injection (cell therapy)

In 2008, Centeno et al reported, for the first time a case showed promising functional outcome after injection of expanded autologous MSCs in a knee joint. MRI confirmed an increase in cartilage and meniscus volume [32]. A complete cartilage coverage has

been reported in nine patients in the cell-recipient group in a double blind randomized trial [33]. However, the injected MSCs adhered to synovial tissues may increase the risk of synovial proliferation. In addition, cellular adhesion to synovial tissue may result in less cellular adherence to the chondral defects. Early clinical data suggests bone marrow aspirate concentrate (BMAC) may help stimulate a hyaline cartilage repair through both chondrocyte differentiation of MSCs and paracrine functions [34]. Soler et al. obtained excellent clinical and quantitative MRI outcome measures with no adverse events after intra-articular injection of 40 × 10⁶ of autologous expanded BM-MSCs [35].

In a systematic review (11 studies, 8 with chondral lesions), the age range of patients with chondral lesions in the knee treated with BMAC injection treatment was 17 and 58 years [3,15,36,37]. Although, many authors suggested the good outcome with injectable treatment but current evidence does not support the use of intra-articular MSCs for improving cartilage repair in knee osteoarthritis [38]. The safety of MSCs has been in question. Centeno et al reported joint swelling and pain in 5.3% patients [32] while Gobbi et al reported 2 patients with joint stiffness [37]. The contradictory evidence of injection treatment suggests that scaffolds may be required for the regeneration of cartilage and could act as a cell carrier.

Surgical implantation (cell scaffold combination)

Kuroda et al (2007) first assessed the effectiveness of autologous MSC, embedded within a collagen polymer, to repair a full-thickness articular cartilage defect (20 × 30 mm) in the medial femoral condyle of a 31-year old athlete. The implant was covered with an autologous periosteal flap. After a year, a hyaline-like cartilage tissue had formed on histological examination. The clinical symptoms had improved significantly and attained his previous activity level [39]. Wakitani et al. presented their results after the treatment of nine cartilage defects (patella-femoral) in three patients. They introduced BM-MSCs on collagen gel covered with periosteum in one case and synovium in the other two cases. Clinical improvement was reported at 7–21 months [40].

De Windt reported one surgery two-cell technique (combined allogenic MSC with recycled autologous chondrocytes and native pericellular matrix) in knees. At 12 months, all (10 patients between age 18–45 years) showed significant good functional outcome. Histological analysis indicated hyaline-like cartilage with a high concentration of proteoglycans and type II collagen [41]. Emadedin et al randomized, triple-blind, placebo-controlled RCT demonstrated the safety and efficacy of a single intra-articular implantation of 40 × 10⁶ autologous MSCs in patients with knee OA. The procedure provided significant and clinically relevant pain relief over 6 months versus placebo [42].

Gobbi et al. prospectively analyzed at five years, 50 (fifty) physically active patients (mean age, 45 years) with grade IV cartilage injury of the knee (lesion size, 1.5–24 cm²) who were treated with HA (Hyaluronic Acid)-BMAC or microfracture. The outcome scores were significantly improved in both groups at 2 years (P < .001). HA-BMAC implantation for cartilage repair can lead to successful medium-term outcomes independent of age or lesion size [37]. They observed hyaline-like cartilage in 80% of patients seen on magnetic resonance (MRI) imaging.

Haleem et al reported 5 (five) patients treated with MSCs transplanted on a scaffold of platelet-rich fibrin glue (PR-FG) for chondral defects of knees. All patients had successful outcome. They concluded that PR-FG might be an ideal MSC scaffold since platelets (secretory granules) contain both TGF-1 and IGF-1 [43]. Wakitani et al in their longest follow up of 10 years in patients with transplanted autologous BMSC reflected that this is a safe procedure without any tumor formation [44]. In the last few years, the high quality of evidence to support MSC therapy has emerged but further refinement of methodology will be necessary to support its routine clinical use [45].

Future Directions

MSC Exosomes

Exosomes are extracellular vesicles secreted through the fusion of multivesicular endosomes with the cell membrane. MSC exosomes provide new perspectives for the development of cell-free and ready-to-use therapy for treatment of cartilage lesions and OA. Zhang et al first reported the effects of human embryonic MSC (EMSC) exosomes on cartilage repair [46].

Genetic engineering

Gene transfer to cartilage defects can be achieved by either direct vector administration to cells located at or surrounding the defects, or by transplantation of genetically modified chondrogenic cells into the defect [47]. Despite promising results, for an effective gene-based therapy of cartilage defects many barriers need to be crossed before clinical translation in patients.



Epigenetics

Epigenetic gene regulation commonly refers to a heritable and long-lasting process by which gene expression is handled at chromatin level without alterations in DNA sequence i.e. post-genetic or non-genetic regulation [17,48,49]. The epigenetics of MSCs is an intriguing area of investigation holding great promise for both basic and applied researches in tissue engineering [48].

Conclusion

Cell-based therapies are emerging as a means to regenerate cartilage. However, there are still unknown mechanisms of tissue repair using MSCs; for instance, it is not yet known whether the transplanted MSCs directly fill the lesion and regenerate the defect in the AC or they indirectly stimulate through their paracrine functions. At present, MSC-based therapies are not suitable for regeneration of large cartilage lesions in severe OA patients, the criteria of optimal scaffold, cell dose, injected times, and intervals are not definite. The challenge for the future consists in addressing specific researches providing more insights into the MSC exosomes, genetic engineering and epigenetic regulatory mechanisms governing MSC biology.

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