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Knee Cartilage Regeneration with Umbilical Cord Mesenchymal Stem Cells Embedded in Collagen Scaffold Using Dry Arthroscopy Technique

B. Sadlik, G. Jaroslawski, D. Gladysz, M. Puszkarz, M. Markowska, K. Pawelec, D. Boruczkowski, and T. Oldak

Abstract

Articular cartilage injuries lead to progressive degeneration of the joint with subsequent progression to osteoarthritis, which currently becomes a serious health and economic issue. Due to limited capability for self-regeneration, cartilage repair remains a challenge for the present-day orthopedics. Currently, available therapeutic methods fail to provide satisfactory results. A search for other strategies that could regenerate a hyaline-like tissue with a durable effect and adequate mechanical properties is underway. Tissue engineering strategies comprise the use of an appropriately chosen scaffold in combination with seeding cells. Mesenchymal stem cells (MSC) provide an interesting new option in regenerative medicine with solid preclinical data and first promising clinical results. They act not only through direct cartilage formation, but also due to paracrine effects, such as releasing trophic factors, anti-inflammatory cytokines, and promoting angiogenesis. The MSC can be applied in an allogeneic setting without eliciting a host immune response. Out of the various available sources, MSC derived from Wharton's jelly of an umbilical cord seem to have many advantages over their counterparts. This article details a novel, single-staged, and minimally invasive technique for cartilage repair that involves dry arthroscopic implantation of scaffold-embedded allogenic mesenchymal stem cells isolated from umbilical cord Wharton's jelly.

B. Sadlik (✉), G. Jaroslawski, and M. Puszkarz
Biological Joint Reconstruction Department, St Luke's Hospital, Bielsko-Biala 43-300, Poland
e-mail: sadlik@lukasza.pl

D. Gladysz, M. Markowska, D. Boruczkowski, and T. Oldak
Polish Stem Cell Bank, Warsaw, Poland

K. Pawelec
Department of Pediatric Hematology and Oncology, Warsaw Medical University, Warsaw, Poland

Keywords
Articular cartilage • Cartilage reconstruction • Chondral defect • Matrix aided implantation • Mesenchymal stem cells • Tissue engineering • Wharton's jelly

1 Introduction

Cartilage degeneration is a significant and growing problem of modern orthopedics that creates a serious economic burden and becomes a growing public health issue. Even minor cartilage defects could lead to bone-on-bone contact and knee malalignment resulting in osteoarthritis, which is a leading cause of pain and disability among older population (Lawrence et al. 2008). With longer life expectancy, aging population, and increasing obesity, the prevalence of osteoarthritis appreciably rises (Cross et al. 2014). The goal of the successful regeneration is to preserve natural abilities of native cartilage including mechanical and functional properties of the knee as a weight-bearing joint. Moreover, the desired effect should be long-lasting. Achieving a successful reconstruction of cartilage tissue still remains a challenge due to its avascular and aneural nature that highly limits intrinsic regenerative capacity (Leijten et al. 2013). Several therapeutic approaches have been carried out to address cartilage regeneration, but they fail to fulfill clinical needs. Due to dissatisfying results, there is a need of employing novel, more effective therapeutic techniques. Recent advances in stem cell engineering have led to clinical application of various cell types with different methodological approaches and promising, yet conflicting results. Herein we present a novel method for cartilage regeneration with umbilical cord Wharton's jelly-derived mesenchymal stem cells, a collagen scaffold, and dry arthroscopy technique.

2 Scientific Rationale

2.1 Mesenchymal Stem Cells – Definition and Properties

Mesenchymal stem cells (MSC) are considered to be promising in tissue engineering. According to the guidelines the International Society for Cellular Therapy, MSC ought to be plastic-adherent during standardized culture conditions, express CD105, CD73, and CD90, be negative regarding lineage antigens (CD45, CD34, CD14, CD19, and HLA-DR), and be able to differentiate into osteoblasts, adipocytes, and chondroblasts (Dominici et al. 2006). They are considered weekly or non-immunogenic due to low expression of class I HLA and no expression of class II HLA and co-stimulatory molecules, and thus can be applied in an allogeneic setting (Law and Chaudhuri 2013). A study on MSC immunogenicity revealed that, when given intra-articularly to 5-year-old mares as an autologous, allogeneic, or even xenogeneic material, they elicit a host immune response only after re-exposure to xenogeneic cells. No arthroscopic or histologic changes in synovium have been detected (Pigott et al. 2013). Due to low immunogenicity, MSC in combination with biomaterials could constitute a tissue-engineered product available for off-the-shelf application.

2.2 Wharton's Jelly as Abundant Source of Mesenchymal Stem Cells

Wharton's jelly is a gelatinous substance composed out of high amount of extracellular matrix

that surrounds and protects cord blood vessels (Wang et al. 2004). Although MSC can be isolated from various sources including bone marrow and adipose tissue, Wharton's jelly-derived MSC (WJ-MSC) seem to be a preferable source because of easiness and safety of the harvesting procedure as well as a rich number of cells contained in the umbilical cord. WJ-MSC have high proliferation and differentiation capabilities, superior to adult stem cell sources, and characteristics close to embryo-derived stem cells, but with no risk of potential tumorigenesis. In contrast, regenerative capabilities of adult stem cell sources seem to decrease with donor's age (Beane et al. 2014). There is evidence that WJ-MSC are genetically stable and retain their immature immunophenotype, functional features, and immunomodulatory properties during long-lasting ex-vivo expansion (Chen et al. 2014; La Rocca et al. 2013). Moreover, in comparison to other sources, MSC derived from neonatal tissues are immunologically privileged with high expression of immunomodulatory factors and low expression of class I HLA (Deuse et al. 2011).

2.3 Mechanism of Action and Preclinical Data on Cartilage Regeneration

MSC can influence cartilage regeneration trough differentiation into chondrogenic lineage, inducing proliferation and differentiation of chondrocyte progenitors, and modifying reaction of endogenous cells. Paracrine mechanisms also play an important role in enhanced regeneration, through release of trophic factors and exertion of anti-inflammatory effects (Toh et al. 2016). WJ-MSC down-regulate expression of matrix-degrading enzymes released from synovium and avert cartilage damage in the xenogeneic animal model (Saulnier et al. 2015). The WJ-MSC potential in treating cartilage lesions and the ability to differentiate into chondrogenic lineage was confirmed in a study with type 1 collagen hydrogel as a scaffold (Chen et al. 2013). These investigators have demonstrated the expression of cartilage-specific matrix proteins and a chondrogenic transcription factor after incubation in chondrogenic medium. The WJ-MSC capabilities to regenerate cartilage are comparable, or even superior, to other sources with the advantage of maintaining the immune-privileged characteristics (Danišovič et al. 2016; Liu et al. 2012; Wang et al. 2009). Both WJ-MSC and MSC derived from adult tissues are found compatible with various scaffolds, which leads to encouraging effects (Musumeci et al. 2014). Promising results regarding cartilage repair have already been reported in animal models and first clinical studies (Filardo et al. 2013).

3 WJ-MSC Procedures

3.1 Cell Culture Preparation

The therapeutic medical experiment consisting of tissue cultures of samples taken from human umbilical cord was approved by a local Bioethics Committee. Informed consent was obtained for sample collection from donor mothers scheduled for natural or cesarean delivery. Umbilical cord tissue was transported in a controlled temperature and processed within 48 h after delivery. Tissue fragments were washed in sterile saline with the addition of antibiotic/antimycotic solution, cut into 2 cm pieces (Fig. 1a), and had blood vessels removed (Fig. 1b). Subsequently, Wharton's jelly was sliced into 2 cm^3 scraps (Fig. 1c) that were placed in a flask for MSC growth in a xeno-free medium supplemented with antibiotics (Fig. 1d). Cell cultures were incubated in in the air with 5% CO_2 at 37 °C. After 2–3 weeks, tissue explants were removed and adherent cells were passaged until reaching 90% of confluence (Fig. 1e). Cells were reseeded at 1.2×10^4cells/cm^2 in culture flasks for further

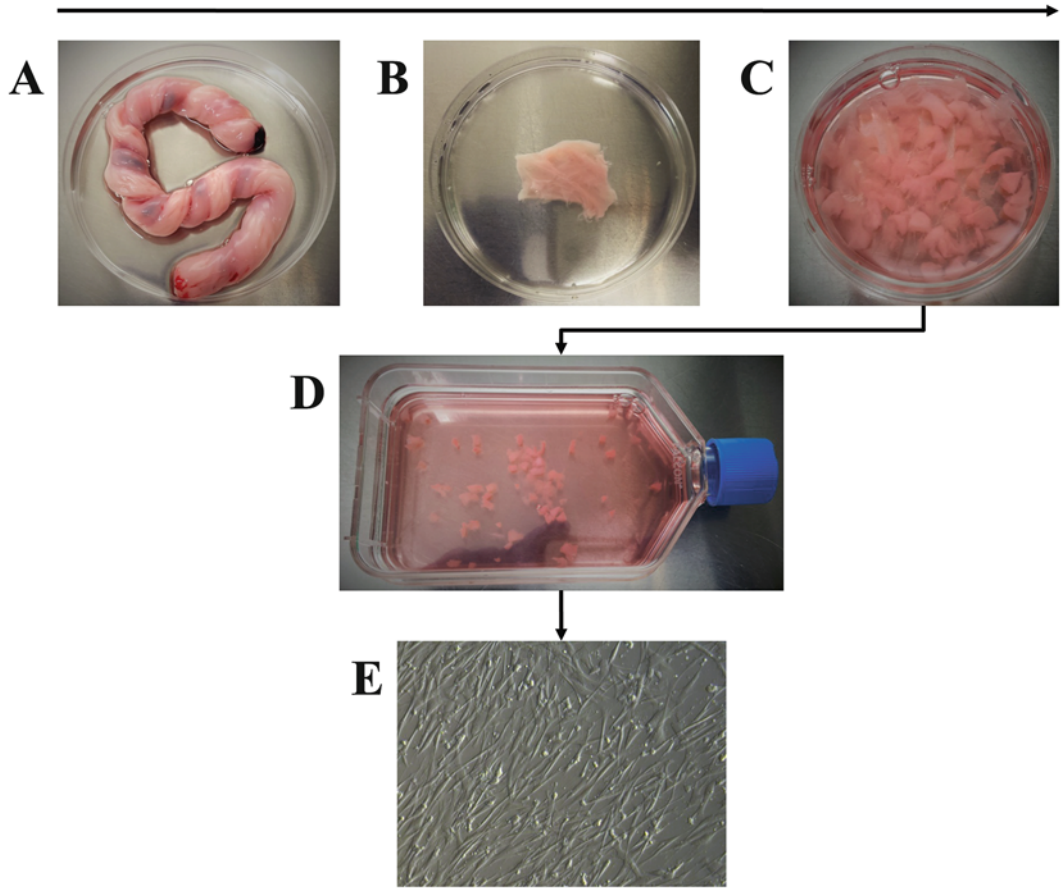

Fig. 1 Key stages of cell culturing: (**a**) – human umbilical cord (~10 cm); (**b**) – sectioned piece of Wharton's jelly after washing in sterile saline with antibiotic/antimycotic solution; (**c**) sectioned piece of Wharton's jelly sliced into 2 cm^3 fragments; (**d**) – scraps placed in a flask with xeno-free medium for mesenchymal stem cells (MSC) expansion; and (**e**) – a contrast image of Wharton's jelly MSC upon reaching 90% of confluence

expansion. Viability of expanded WJ-MSC was determined by the trypan blue exclusion in hemocytometer, and the cells' characteristics were confirmed with immunophenotyping by the presence or absence of surface markers (CD73-, CD90-, and CD105-positive; and CD34-, CD14-, CD19-, CD45, and HLA DR-negative). A reference sample of WJ-MSC was incubated with antibodies for 30 min in darkness and washed with cell wash solution. Next, cells were resuspended in cell fix solution and checked using flow cytometry with fluorescein isothiocyanate (FITC) and phycoerythrin (PE) conjugated to anti-mouse IgG1 antibody as control. The WJ-MSC intended for a therapeutic use were suspended in a mixture of human albumin in a presence of 10% dimethyl sulfoxide (DMSO), and transferred into freezing bags, which were placed in cell containers and cooled in a controlled rate freezer. After the freezing process, cells were stored in liquid nitrogen at −195 °C.

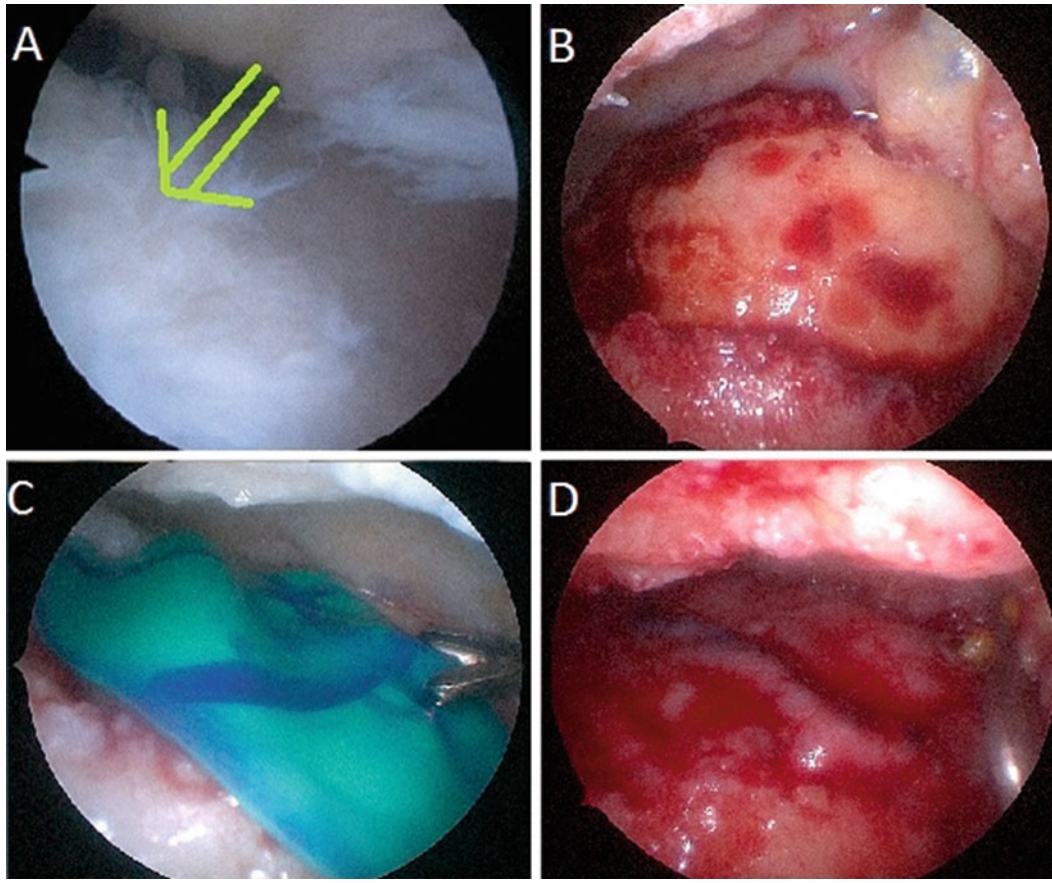

Fig. 2 The WJ-MSC procedure. Arthroscopic visualization of the right medial compartment through anterolateral working portal. (**a**) – full-thickness femoral condyle chondral injury (*arrow*); (**b**, **c**, and **d**) – retracting plate positioned to draw back capsule and adjacent synovial tissue to improve access to the chondral lesion: (**b**) – a view after preparing a chondral defect with vertical walls, (**c**) – a rubber templet positioned in the defect to check the shape and size of the scaffold, (**d**) – final position of a collagen scaffold embedded with WJ-MSC and covered with the fibrin glue to enhance stability of WJ-MSC in the graft

3.2 Thawing and Washing Procedure

The thawing procedure was conducted 30 min before the estimated implantation time. The freezing bag with WJ-MSC was quickly warmed up in a water bath at 37 °C. The DMSO cryoprotectant was washed out from WJ-MSC in a two-step dilution method with saline. The WJ-MSC were transferred to a sterile conical tube and suspended in 50 ml of saline. The cells were collected by centrifugation at 300 × *g* for 7 min at 22 °C. The supernatant was aspirated, and the pellet fraction was resuspended in 50 ml of saline and centrifuged again. The pellet fraction consisting of WJ-MSC was resuspended in 1 ml of saline. Finally, a suspension of WJ-MSC was transferred into a sterile syringe.

3.3 Patient Positioning and Arthroscopic Chondral Defect Preparation

The patient is positioned supine as for the standard knee arthroscopy. The procedure is typically performed under general or spinal anesthesia. A diagnostic arthroscopy is performed to visualize the entire cartilage injury and to characterize the lesions suitable for repair (Fig. 2a). Beside standard curettes, specially designed instruments

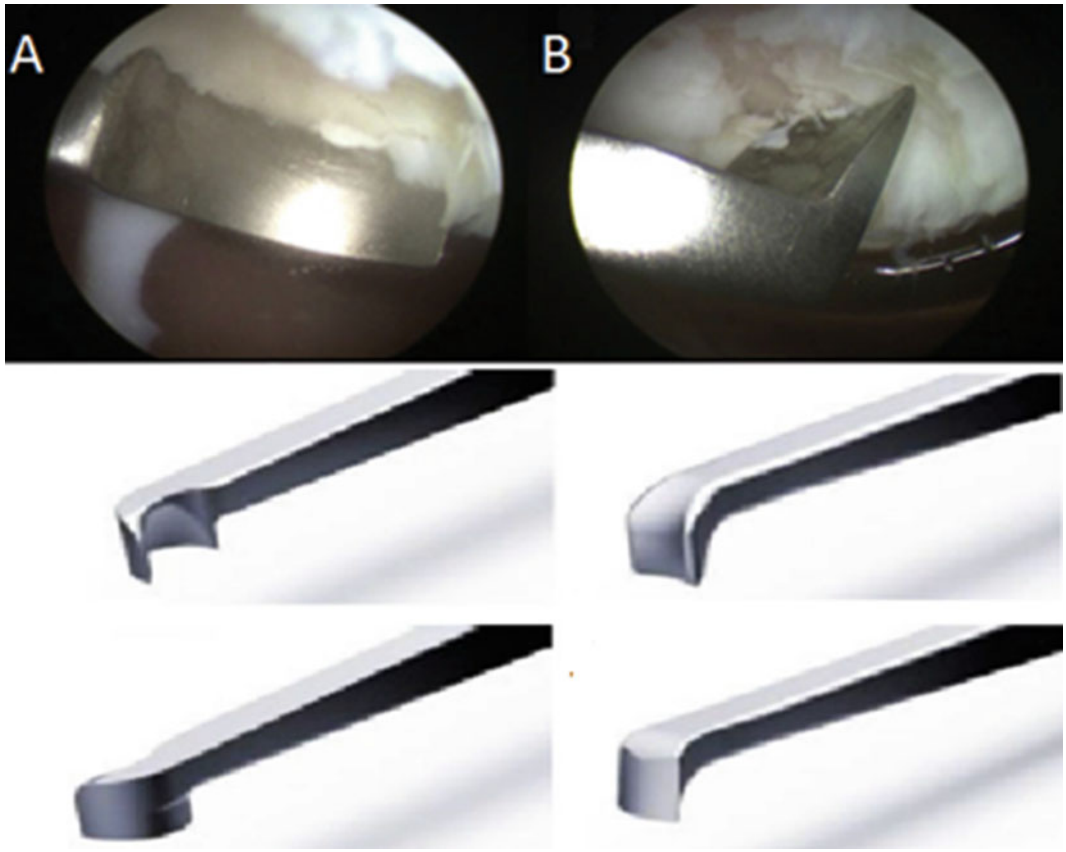

Fig. 3 Chondrectomes: instruments designed for arthroscopic removal of damaged cartilage and for creation of vertical ring surrounding the defected cartilage

(Chondrectomes Set, ATMED – Z. Rafalski, Katowice, Poland) may help optimize access and curettage of cartilage lesions, particularly when a parallel approach to the chondral defect is required (Fig. 3a, b). Loose chondral tissue associated with the lesion is excised, and curettes are used to create a contained lesion on the articular cartilage surrounded with a vertical border. Care is taken to remove the calcified cartilage layer overlying the subchondral bone, without violating the subchondral plate.

3.4 Wharton's Jelly-Derived Mesenchymal Stem Cell (WJ-MSC) Scaffold Preparation

A template created from aluminum foil or sterile latex dental dam (Sanctuary Dental Dam Systems; Ipoh, Malaysia) is inserted into the chondral defect to confirm the correct size and shape if needed (Fig. 2c). According to the defect dimensions, an appropriately sized implant is fashioned from the porcine type I/II collagen matrix (Chondrogide; Geistlich Biomaterials, Wolhusen, Switzerland) (Fig. 2d). The trimmed scaffold is wetted using saline and subsequently immersed with a suspension of WJ-MSC for 5 min, creating a malleable implant.

4 Dry Arthroscopic Implantation of WJ-MSC Embedded Scaffold

During dry arthroscopic visualization of the lesion, exposure is manageable by the retraction of the joint capsule and synovium, using a specially designed retracting system (Arthroscopic

Retracting System; ATMED – Z. Rafalski, Katowice, Poland) (Fig. 2b). When arthroscopic fluid is sucked from the working articular space, a skid or halfpipe has to be placed in the working portal to maintain an open gate to equalize the pressure in the joint. When undertaking an arthroscopic cartilage repair of a patella, femoral condyle, or tibial plateau, the entire cartilage defect should be visualized. The WJ-MSC embedded scaffold is inserted into the defect through the halfpipe and placed in the working portal with the use of a special inserter named 'fork'. Subsequently, the matrix is gently slid off from the 'fork' into the defect and kept in place, while an arthroscopic hook is introduced from the opposite portal to fit the implant into the prepared bed by pressing it (Fig. 2d). A fibrin glue (Tisseel Lyo; Baxter Healthcare Corp., Westlake Village, CA) is applied for covering the matrix to improve stability and to prevent WJ-MSC from migration into the synovial fluid. When the implant stability is confirmed, the wounds are closed and the joint is immobilized in the brace on the operating table.

4.1 Postoperative Rehabilitation and MRI Monitoring

The knee is immobilized for 5 days after the surgery to maintain a stable fibrin clot fully protecting biological implant. During this period patient is provided with muscle isokinetic training on the operated limb and exercise on the other body parts 4 times a day. A proper use of crutches is trained during first days after surgery. On the sixth day, the first passive mobilization with retracting the injured compartment is applied, followed by further passive mobilization 2–3 times a day. The MRI examination is carried out after the third week postoperatively to check for the graft position. When the examination confirms the proper status of the graft, more intensive mobilization is recommended. Weight-bearing is restricted for 3–6 weeks depending on the localization and size of the implanted scaffold. After 6 weeks, patient starts with a weight-bearing training of muscle strength, stability, and proprioception. Within the next 2–4 weeks, patient progresses to normal walk pattern without crutches. After 3 months, unrestricted physical activity is allowed. MRI scans are performed 1.5, 6, and 12 months after the surgery to observe graft incorporation and rebuilding. The early state of the chondral defect regeneration, with comparison to preoperative state, of the lateral compartment of a knee is presented in Fig. 4.

5 Discussion

Mesenchymal stem cells, in combination with biomaterials, carry a great potential that has already been proven in animal studies and first clinical applications. However, application of WJ-MSC have not yet been described in a clinical setting. With hitherto unsatisfactory results and a constantly increasing need for unicompartmental or total knee replacement surgery in case of large chondral lesions or osteoarthritic changes (Ackerman et al. 2016), a search for novel treatment methods has been underway. Tissue engineering strategies could constitute a major upturn in cartilage repair approaches. The key for successful articular cartilage regeneration lies in carefully selected components of tissue engineering: a scaffold with an adequate biomechanical properties compatible with non-immunogenic seeding cells with high chondrogenic potential, and a surgical technique adjusted for a precise graft implantation. Dry arthroscopy technique, along with scaffold and bone marrow-derived MSC, have been previously applied for the reconstruction of cartilage with good results (Gobbi et al. 2016, Gobbi et al. 2014). This technique requires a precise, minimally invasive surgery (Whyte et al. 2016; Sadlik and Wiewiorski 2014). The WJ-MSC procedure can be carried out regardless of the exact site of the knee cartilage injury due to the use of retracting plate, chondrectomes, and a halfpipe. The WJ-MSC is a preferable source of stem cells, with promising preclinical data. We propose a novel single-stage technique of cartilage regeneration that includes a suitably selected scaffold for WJ-MSC and dry arthroscopy technique, along with careful monitoring and controlled

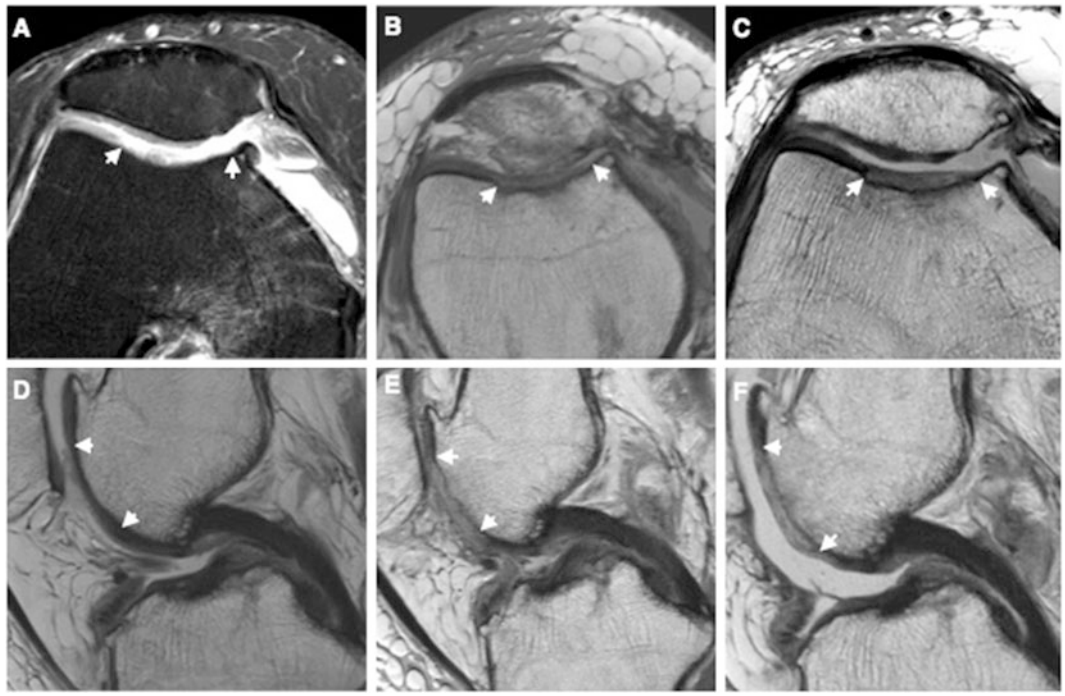

Fig. 4 Proton density MRI of a repaired defect (*arrows*): axial scans (*upper row*) and sagittal scans (*lower row*). (**a** and **d** – patellofemoral joint preoperatively: irregular cartilage defect of the trochlea (grade 3/4 according to the International Cartilage Repair Society (ICRS) scale of osteochondritis dissecan lesions), defect of medial femoral condyle, degenerative marginal spiking of the patellofemoral joint; (**b** and **e**) – regenerative tissue after the dry arthroscopic implantation of WJ-MSC embedded scaffold is well visible 3 weeks postoperatively (*green arrows*); (**c** and **f**) – after 6 weeks, regenerative tissue abounds and is integrated with the surrounding cartilage and subchondral lamina

rehabilitation. It is a challenging approach for cartilage regeneration, especially in patients with poor biological self-regenerative ability. MRI scans were done to assess the potential side effects and to evaluate the safety of proposed strategy. Synovial proliferation is considered a potential adverse effect of MSC-induced cartilage regeneration, as a large part of stem cells home in on the synovium rather than cartilage tissue. In view of such a risk, Koga et al. (2008) have suggested that MSC should be in a direct contact with cartilage surface for about 10 min. However, their study was based on the synovial MSC, which could have influenced the results. In the period from July 2015 to November 2016, we performed five surgeries, all approved by a local Bioethics Committee (permit no. 2015/06/25/1 BIL), in patients who had not benefited from standard therapies for a knee cartilage injury. We did not observe infections, excessive synovial proliferation, tumor formation, graft rejection, graft versus host reaction, or any other adverse effects. All patients also benefited from a significant knee pain reduction. However, long-term follow-up is required to assess the quality of newly formed cartilage and clinical outcomes.

This study has several limitations. A small number of patients in the preliminary phase of the study was a consequence of cautious patient qualification due to previous poor clinical experience with WJ-MSC application. After the promising results had been obtained with the very first cases and no apparent adverse events, the patient recruitment became more courageous. Another limitation was the lack of a control group, which was due to the fact that the study had been designed to confirm the efficiency of a new

method on the basis of clinical results and MRI examinations. Moreover, it was a therapeutic experiment with the goal to achieve clinical benefits in patients who had not improved during standard therapy; thus a control group was unavailable. All enrolled patients met the criteria for total or unicompartmental knee alloplasty. The WJ-MSC provided the patients a chance to postpone the major surgical intervention and to substitute it for a minimally invasive treatment. The method described herein may be an important contribution to the advancement of tissue engineering aiming at articular cartilage repair, especially with regard to joint degenerative changes in elderly patients.

6 Conclusions

The aim of stem cell-enhanced cartilage repair is to acquire a hyalin cartilage-like tissue that is indistinguishable from the native one in both functional properties and histological structure. The WJ-MSC are promising seed cell candidates for tissue engineering and cell-based cartilage regeneration. Combined with an adequate scaffold, appropriate surgical technique, and a careful rehabilitation, they may hold the key for successful cartilage regeneration, particularly in elderly patients with poor intrinsic MSC regenerative potential. In this study we demonstrate that WJ-MSC could be used to induce regeneration of cartilage. To the best of our knowledge, this is the first presentation of clinical application of scaffold-embedded WJ-MSC through dry arthroscopy.

Supplementary Data Video presentation of arthroscopic cartilage repair of lateral compartment - https://www.youtube.com/watch?v=FPq_JU1DOskandfeature=youtu.be

Conflicts of Interest The authors declare no conflict of interest in relation to this article.

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