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# A Novel Method for the Knee Cartilage Regeneration With Umbilical Cord Mesenchymal Stem Cells Embedded in a Scaffold Using Dry Arthroscopy Technique.

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Articular cartilage injuries lead to progressive degeneration of the joint with subsequent progression to osteoarthritis that 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 durable effect and adequate mechanical properties is underway. Tissue engineering strategies comprise 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. MSC can be applied in an allogeneic setting without eliciting host immune response. Out of the various available sources, MSC derived from a part of umbilical cord called Wharton's jelly, 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 (DASI WJ-MSC).

Keywords: DASI WJ-MSC, MSC, Stem Cells, Wharton's Jelly, Tissue Engineering, Cartilage Reconstruction, Cartilage Defect, Chondral Defect, Orthopedics, Matrix Aided Implantation

## 1. Introduction

Cartilage degeneration is a significant and growing problem of the 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 the leading cause of pain and disability among older population (Lawrence et al. 2008). With longer life expectancy, aging population and increasing obesity, incidence and prevalence of osteoarthritis rise significantly (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 have to 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, however they were not fully satisfactory 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 (DASI WJ-MSC).

# 2. Scientific Rationale

## 2.1. Mesenchymal Stem Cells – Definition and Properties

Mesenchymal stem cells (MSC) are considered to be promising in tissue engineering. They have been defined by International Society for Cellular Therapy. According to the guidelines they must 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 and of class II HLA and co-stimulatory molecules, and thus can be applied in an allogeneic setting (Law & 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 host immune response only after re-exposure to xenogeneic cells. No arthroscopic or histologic changes in synovium were 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 an 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 (UC). 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 age (Beane et al. 2014). There are evidences 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. 2014). Moreover, in comparison to other sources, MSC derived from neonatal tissues are immunologically privileged with high expression of immunomodulatory factors and lower 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 play an important role as well through enhancing regeneration, releasing trophic factors and exerting anti-inflammatory effect (Toh et al. 2016). WJ-MSC were found to down-regulate expression of matrix-degrading enzymes released from synovium and avert cartilage damage in xenogeneic animal model (Saulnier et al. 2015). WJ-MSC potential in treating cartilage lesions and its ability to differentiate into chondrogenic lineage was confirmed in a study with type 1 collagen hydrogel as a scaffold. The investigators proved expression of cartilage-specific matrix proteins and chondrogenic transcription factor after incubation in chondrogenic medium (Chen et al. 2013).WJ-MSC capabilities to regenerate cartilage are comparable or even superior to other sources with an advantage of maintaining their immune privileged characteristics (Danišovič et al. 2016; Liu et al. 2012; Wang et al. 2009). Both WJ-MSC and MSC derived from adult tissues were found compatible with various scaffolds resulting in 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. DASI WJ-MSC Procedure

## 3.1. Cell Culture Preparation

After obtaining a Bioethical Committee agreement for a therapeutic medical experiment procedure, cell cultures are established with the use of human UC. Samples of UC are collected after an informed consent of donor-mother after both cesarean section and natural delivery. UC tissues are transported in a controlled temperature and processed within 48 hours after the delivery. UC fragments are washed in a sterile saline with antibiotic/antimycotic solution, cut into 2cm pieces (Fig.1A) and have blood vessels removed (Fig.1B). Subsequently Wharton's jelly is sliced into $2cm^3$ scraps (Fig.1C) that are placed in a flask and grown in a xeno-free medium for MSC with supplements and antibiotics (Fig. 1D). Cell cultures are incubated at 37°C in the atmosphere of 5% $CO_2$ in the air. After 2-3 weeks of culture, tissue explants are removed and adherent cells are passaged upon reaching 90% confluence (Fig.1E). Cells are reseeded at $1.2x10^4 cells/cm^2$ in culture flasks T75 for further expansion. Viability of expanded WJ−MSC is determined by the trypan blue exclusion in hemocytometer, and their characteristics is confirmed with immunophenotyping by the presence or absence of characteristic surface markers (CD73-, CD90-, CD105-positive and CD34-, CD14-, CD19-, CD45, HLA DR-negative). A reference sample of WJ-MSC is incubated with antibodies for 30 minutes in darkness and washed with Cell Wash solution. After that, cells are resuspended in Cell Fix solution and analyzed using flow cytometer with mouse IgG1 FITC and IgG1 PE as a control. WJ−MSC intended for therapeutic use are suspended in a mixture of human albumin in a presence of 10% dimethyl sulfoxide (DMSO), and transferred into freezing bags, which are placed in cell containers and cooled in a controlled rate freezer. After the freezing process, the cells are stored in a liquid nitrogen (-195°C).

## 3.2. Thawing and Washing Procedure

The procedure of thawing is conducted about 30 minutes before the estimated implantation time. The freezing bag with WJ−MSC is taken from the cell container after removing it from a liquid nitrogen, and quickly warmed-up in a water bath at 37°C. DMSO is washed out from WJ−MSC in a 2-step dilution method with saline for the cryoprotectant removal. After warming up, WJ−MSC are transferred from the freezing bag to a sterile conical tube and suspended in 50 ml of saline. Cells are collected by centrifugation at 300G for 7 minutes at 22°C. Supernatant is aspirated and the pellet is resuspended in saline. Subsequently, WJ−MSC are suspended in 50 ml of saline and cells are again collected by centrifugation at 300G for 7 minutes at 22°C. As before, supernatant is aspirated and pellet is resuspended in 1 ml of saline. Afterwards the suspension of WJ−MSC is transferred to 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 entire cartilage injury and characterize those lesions suitable for repair (Fig. 2A). Beside standard curettes, specially designed instruments may help to optimize access and curettage of cartilage lesions, particularly in cases of parallel approach to the chondral defect (Chondrectomes Set, ATMED - Z.Rafalski, Katowice, Poland; Fig. 3A and 3B). Loose chondral tissue associated with the lesion is excised, and curettes are used to create a contained lesion on articular cartilage with circumferential vertical walls. Care is taken to remove the calcified cartilage layer overlying the subchondral bone, without violating the subchondral plate.

## 3.4. WJ-MSC Scaffold Preparation

A template created from aluminium foil or sterile latex dental dam (Sanctuary Dental Dam Systems, Ipoh, Malaysia) is inserted into the chondral defect to confirm correct sizing and proper 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 suspension of WJ-MSC for 5 minutes, creating a malleable implant.

### 3.5. Dry Arthroscopic Implantation of WJ-MSC Embedded Scaffold

During dry arthroscopic visualization of the lesions, exposure is manageable by the retraction of the joint capsule and synovium, using a specially designed retracting system (Arthroscopic Retracting System, ATMED - Z. Rafalski). When arthroscopic fluid is sucked from the working articular space, a skid or a 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 and/or tibial plateau, the whole cartilage defect after the preparation should be visualized. The WJ-MSC embedded scaffold is inserted into the defect through the skid or the halfpipe, placed in the working portal with the use of a special inserter named “a fork” that allows to gently slide the matrix into the defect and keep it at the place while an arthroscopic hook is introduced from opposite portal, and fits the implant into the prepared bed by pressing it (Fig. 2D). Fibrin glue (Tisseel Lyo, Baxter) is applied for covering matrix to improve stability and to prevent WJ-MSC from migration into the synovial fluid. After positive confirmation of the implant stability, the wounds are closed and the joint is immobilized in the brace on the operating table.

## 4. 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 exercises on the other body parts 4 times a day. Proper using of crutches is trained during first days after surgery. On the 6$^{th}$ day, the first passive mobilization with retracting the injured compartment is applied following passive mobilization 2 or 3 times a day. The MRI examination is carried out after 3$^{rd}$ week postoperatively to check for the graft position and its status. If it 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 the size of the implanted scaffold. After 6 weeks patient starts with weight-bearing training of muscle strength, stability and proprioception. Within the next 2-4 weeks they progress to normal walk pattern without crutches. After 3 months, an unrestricted physical activity is allowed. MRI scans are performed after 6 weeks, 6 and 12 months after the surgery to observe graft incorporation and rebuilding. Early state of the chondral defect regeneration with comparison to preoperative state of the lateral compartment of the knee is presented in Fig. 4.

## 5. Discussion

MSC in combination with biomaterials, carry a great potential that has already been proven in animal studies and first clinical uses, however application of WJ-MSC have not been described in a clinical setting before. With unsatisfactory results of other therapeutic methods and constantly increasing need for a unicompartmental or total knee replacement surgery for big chondral lesions or osteoarthritic changes in the knee (Ackerman et al. 2016), a search of novel treatment methods is needed. Tissue engineering strategies could be a turnover 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 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). It has enabled to carry out a precise and minimally invasive surgery (Whyte et al. 2016; Sadlik & Wiewiorski et al. 2014). The DASI WJ-MSC procedure can be carried out regardless of the site of the knee cartilage injury due to the use of retracting plate, chondrectomes, and halfpipe. WJ-MSC seem to be a preferable source of stem cells with promising preclinical data. We propose a novel single-stage technique of cartilage regeneration that includes WJ-MSC, suitably selected scaffold and dry arthroscopy technique along with careful monitoring and controlled rehabilitation. It is a challenging approach for cartilage regeneration, especially in patients with poor biological self-regenerative ability. MRI scans were done to assess for potential side effect and evaluate safety of proposed strategy. Synovial proliferation is considered as a potential adverse effects of MSC-induced cartilage regeneration as a large part of stem cells home to the synovium rather than cartilage tissue. In view of such a risk, Koga et al. suggests that MSC should have direct contact with cartilage surface for about 10 minutes. However, the above-mentioned study was based on synovial MSC, which could have influence over results (Koga et al. 2008). From July 2015 to November 2016, 5 surgeries were performed for patients who did not benefit from standard therapies with preceding agreement of Bioethical Committee (No 2015/06/25/1 BIL). None mild or severe adverse effects were reported. We did not observe infections, excessive synovial proliferation, tumor formation, graft rejection or graft versus host reaction. All patients are satisfied because of significant knee pain reduction. However, long-term follow-up will need to be assessed taking into account quality of newly formed cartilage and clinical outcomes. The authors are aware of several limitations of the present study. The small number of individuals in the preliminary period of the study is a consequence of a cautious patient qualification due to previous poor clinical experience with WJ-MSC application. After promising results of the very first cases and without any adverse events, the authors have decided to be more courageous in further recruitment. The next limitation is a lack of a control group in relation to fact that the study had been designed to confirm new method efficiency on the basis of clinical results and MRI observations. Moreover, as a therapeutic experiment, the study was aimed at medical benefit of the patients who did not improved after standard therapy and thus a control group was unavailable. All enrolled patients met criteria for the total or unicompartmental knee alloplasty. The DASI WJ-MSC was their chance for postponing the final surgical procedure with minimally invasive treatment. The method described here may be an important contribution to advancement in tissue engineering aiming for articular cartilage repair especially concerning joint degenerative changes of elderly patients.

## 6. Conclusion

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

## 7. Supplementary data

Arthroscopic cartilage repair of lateral compartment is shown in Video 1. https://www.youtube.com/watch?v=FPq_JU1DOsk&feature=youtu.be

## 8. Conflict of interest

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

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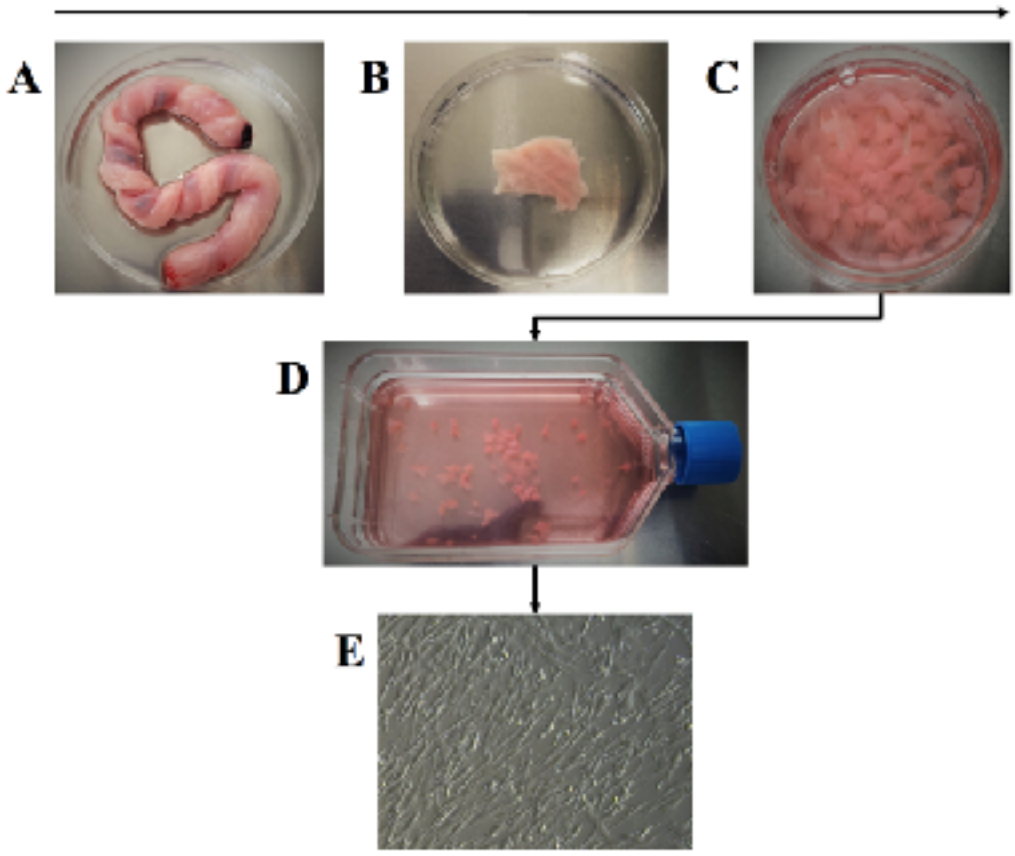


Fig. 1. The key stages of cell culture establishment. (A) human umbilical cord (~10cm); (B) a sectioned piece of Wharton's jelly after washing in sterile saline with antibiotic/antimycotic solution; (C) a sectioned piece of Wharton's jelly sliced into 2 cm3 fragments; (D) the scraps placed in a flask with xeno-free medium for MSC expansion; (E) a contrast image of WJ-MSC upon reaching 90% of confluence.

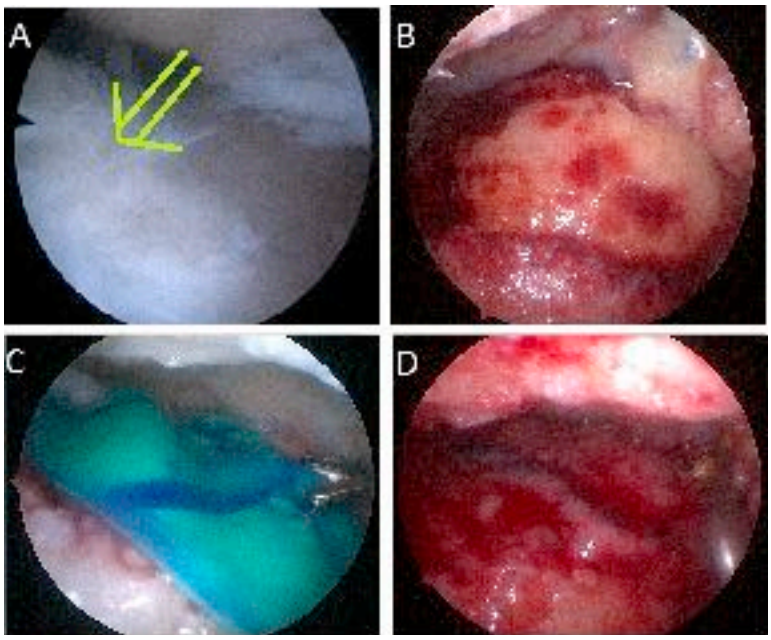


Fig.2. The DASI WJ-MSC procedure; the view of the right medial compartment through anterolateral portal and instrumentation inserted via anteromedial working portal. Arthroscopic visualization of (A) a full-thickness femoral condyle chondral injury (arrow); (B,C,D) - dry arthroscopic view due to retracting plate positioned to retract capsule and adjacent synovial tissue to improve access to the chondral lesions; (B) the view after preparing a chondral defect with vertical walls; (C) a rubber templet is positioned in the defect to finally check the shape and the size of the scaffold; (D) final position of a collagen scaffold embedded with WJ-MSC and covered with the fibrin glue to enhance stability and maintain WJ-MSC in the graft.

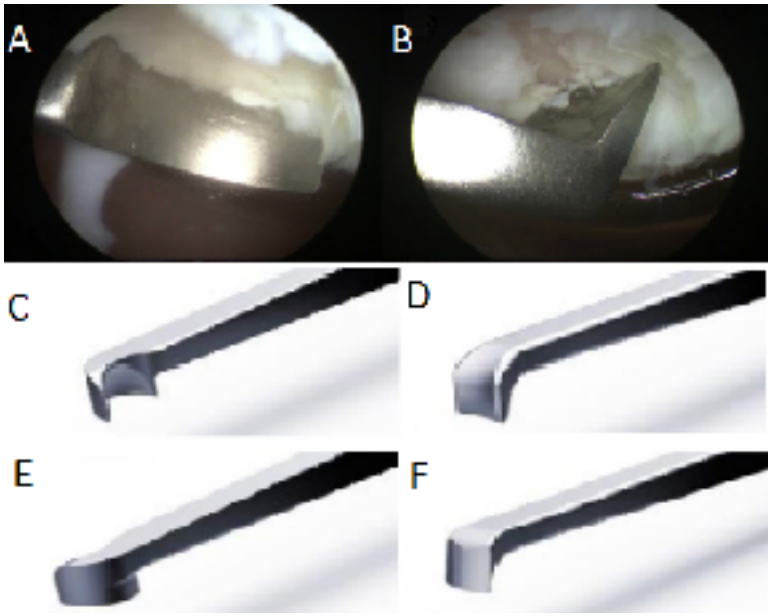


Fig. 3. Chondrectomes - a specially designed instruments for arthroscopic removal of the damaged cartilage that create vertical walls of the defect's surrounding cartilage.

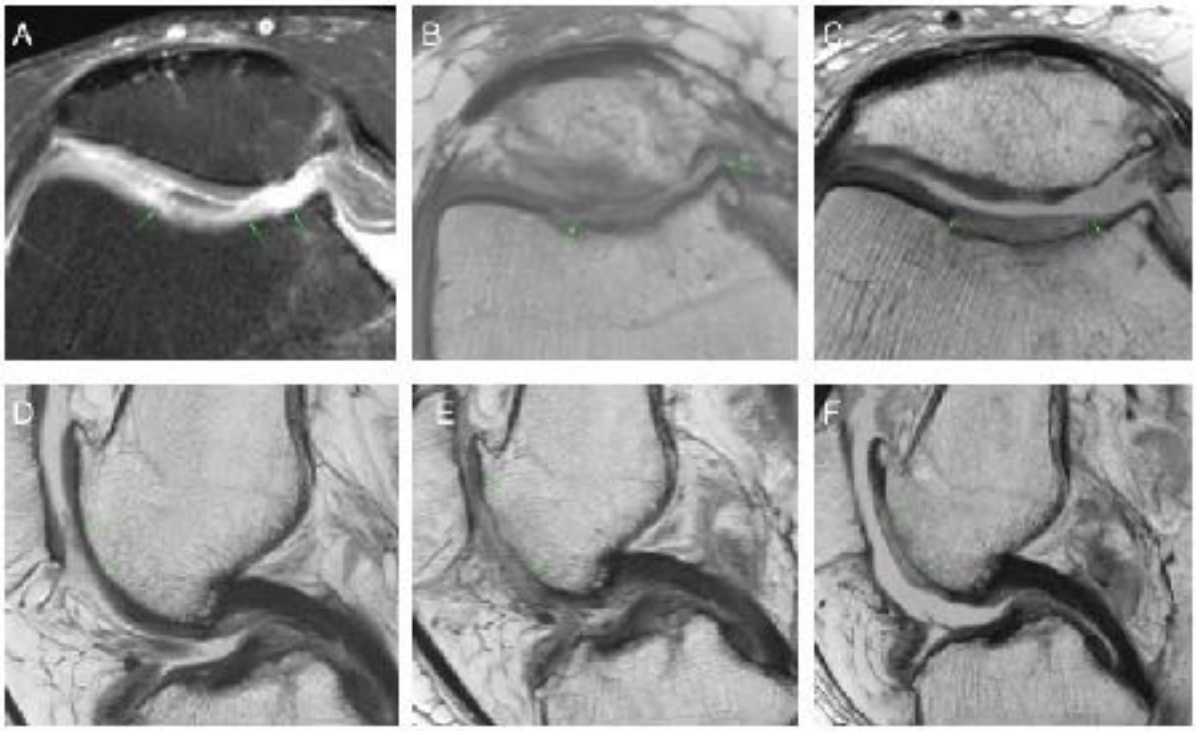


Fig. 4. Proton Density MRI scan of the repaired defect: (A) an axial scan (Fat Saturation) and a saggital scan (D) of the patellofemoral joint preoperatively; irregular cartilage defect grade 3/4 by ICRS of the trochlea and the medial femoral condyle is visible additionally, degenerative marginal spiking of the patellofemoral joint is present; a regenerative tissue after the dry arthroscopic implantation of WJ-MSC embedded scaffold (DASI WJ-MSC) is well visible 3 weeks postoperatively (B, E); after 6 weeks the regenerative tissue seems to be more abundant and well integrated with surrounding cartilage and the subchondral lamina (C, F).