

**“VICTOR BABEȘ” UNIVERSITY OF MEDICINE
AND PHARMACY FROM TIMIȘOARA
FACULTY OF MEDICINE
Department II – General and Dento-maxillary Radiology and
Medical Imaging**

JENEL - MARIAN PĂTRAȘCU



PhD THESIS

**AN IMAGING, HISTOLOGICAL AND FUNCTIONAL STUDY OF
CARTILAGINOUS LESIONS OF THE KNEE, TREATED BY
DIFFERENT SURGICAL METHODS, WITH EMPHASIS ON THE
ROLE OF MRI IN THE DETECTION AND TOPOANATOMICAL
LOCALIZATION OF LESIONS**

A B S T R A C T

Scientific Coordinator:
PROF. UNIV. DR. BRAD SILVIU

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ABSTRACT

INTRODUCTION

In the last decade, the interest in regenerative therapies applied in the field of Orthopedics and Sports Medicine has grown considerably for reasons that are easy to understand. The hypothesis of a regenerative potential is equally attractive for both the patient and the surgeon. Scientific progress in this field is now being made at a faster pace than ever before. The final goal is to assume these therapies as a therapeutic option in the treatment of cartilage lesions, both in acute post-traumatic context and in chronic degenerative context, associated with meniscal lesions with surgical indication of partial meniscectomy.

Currently, there is a consensus regarding the treatment of cartilage damage, namely the preservation of intact tissue wherever possible. However, the impossibility of reconstructing the articular cartilage in complex lesions, both from an anatomical and functional point of view, has led to the continuous search for new management solutions for this pathology.

The motivation of the current study is to bring additional information to the current knowledge in the field of regenerative therapies. The study aims to evaluate the regenerative potential of the microfracture treatment at the level of the induced cartilage defect using non-invasive multiserial imaging methods, with the simulation in experimental conditions of a situation frequently encountered in the clinic and without satisfactory solutions at present. Additionally, it proposes a multi-series method for analysis of the osteochondral fragment using magnetic resonance imaging (MRI) and histological examination.

The primary objectives of the research are the following:

- Demonstration of the regenerative potential of the treatment with microfractures at the level of the cartilage lesion complex with the simulation in the experimental condyle of a situation frequently encountered in the clinic and without satisfactory solutions at the present time. Also, the anatomical specimens in the study lot, were histologically analyzed to reveal modifications and signs of healing process, along with complications.

- Proposing the MRI examination as an extremely efficient technique in evaluating the effect of microfracture therapy in the management of a pathology not compliant with conservative treatment measures, extremely difficult to approach, namely, the evaluation of the articular surface in order to evaluate the remodeling capacity of these therapies on the cartilage.

The secondary objectives of the research are the following:

- Demonstration of the prophylactic role of these therapy in the evolution and progression of chondroplasty towards gonarthrosis at a relatively young age.

The current approach is an original one by carrying out a prospective study that addresses an intensely debated topic in our specialty. The main novelty element consists in the experimental simulation in vivo of a lesion complex frequently encountered in practice, namely: complex knee trauma that includes a cartilage and sometimes associated ligament injuries.

The administration of these therapies was carried out after the surgical treatment of the cartilage lesion, by creating a chondral defect at the level of the rabbit femoral condyle, in order to simulate the operative situation in which, at the time of the surgical intervention for the cartilage lesion, the diagnosis of osteochondral lesion is established. The period of recovery and healing until the moment of harvesting and studying the joints is 6 months. The main method of studying osteochondral remodeling and the healing of cartilage damage is the MRI examination of the rabbit knee with an emphasis on the articular surface in the area of the cartilage defect and obtaining accurate and representative images for the effectiveness of these therapies in the healing and remodeling process of the articular surface. The quality of the joint surface at the end of the healing process is in close biomechanical relationship with the long-term functional result at the level of the affected joint.

The general part deals with the current state of knowledge, presenting the clinical reality in the case of cartilage pathology, with the currently unanimously accepted therapeutic options.

The specialty part includes the actual study carried out to achieve the above-mentioned objectives, as follows:

After the study groups were defined, 21 animals were divided into two groups and the lesions were surgically created, the modifications and the healing process were monitored through histological and imagistic methods.

- the study consisted in the standardization of the MRI examination technique as an extremely valuable method in the detailed description and study of the articular surface, but also in establishing a clear protocol for the study of osteo-cartilaginous parts. The resulting protocol is essential for the evaluation of osteochondral parts from a variety of joint pathologies in order to describe the surface defects and was also used in the following study of the current thesis as an evaluation technique. Thus, a 'mapping' study was carried out on an animal model (rabbit) in which 21 animals divided into two groups were included, in which the complex knee trauma was surgically reproduced and later we used surgical treatment with a regenerative role: microfractures in the subchondral bone, to observe the differences in the healing and remodeling process at the articular surface level. At the end of the 6-month surveillance period, the joints were harvested and analyzed using MRI. The results obtained showed the effectiveness of microfracture therapy in the immediate post-traumatic context.

Therefore, the technique used in this research project, microfractures, falls into the category of regenerative techniques that is intended not only to be minimally invasive for the patient, but also to contribute to the prevention or postponement of complicated and radical interventions, which can open new horizons towards the treatment of degenerative knee pathology from the early stages.

GENERAL PART

The highly specialized connective tissue of diarthrodial joints is called articular cartilage. Its main purpose is to create an articulation surface that is smooth and lubricated and to make it easier to transmit loads that have a low coefficient of friction. Articular cartilage is exposed to a hostile biomechanical environment and lacks blood arteries, lymphatics, and nerves. The ability of articular cartilage to naturally heal and regenerate itself is also essential. In this respect, the articular cartilage's preservation and health are crucial for joint health. It is known that articular cartilage damage contributes significantly to musculoskeletal morbidity.

A condition known as chondromalacia affects the hyaline cartilage that lines the articular areas of bones. The cartilage becomes softer as a result, and is frequently torn or eroded. Microtraumatic damage can cause cartilage to degenerate as well. Chondromalacia can be brought on by repetitive motions that put the joint under compressive tension or by higher loads on the joint. Hyaline cartilage is also affected by aging.

RISK FACTORS FOR CHONDROPATHIES

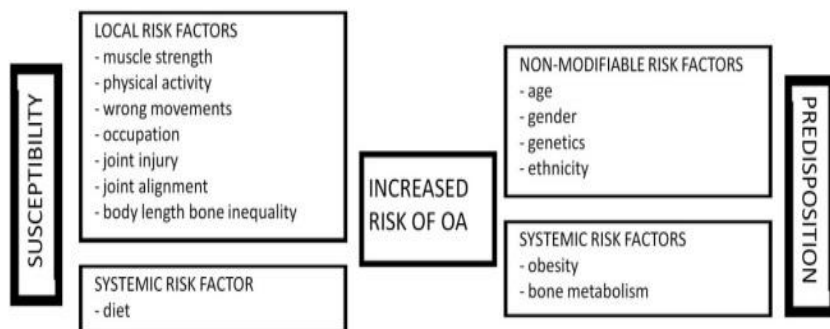


Fig. 4. Major risk factors that increase the susceptibility and predisposition of OA development.

	MR IMAGING	ARTHROSCOPY	MACROSCOPY	SCHEMATIC DRAWING OF THE ARTICULAR CARTILAGE
Grade 0	homogenous and smooth delineation	uniform thickness and intact surface	normal cartilage	
Grade 1	focal areas of hyperintensity with normal contour	softening or swelling of cartilage	focal thickening	
Grade 2	blister-like swelling/fraying of articular cartilage extending to the surface	fragmentation and fissuring within soft areas of articular cartilage	superficial defect(s), less than 50%	
Grade 3	partial thickness cartilage loss with focal ulceration	partial thickness cartilage loss with fibrillation ("crab-meat appearance")	Deep defect(s) more than 50%	
Grade 4	exposed subchondral bone	cartilage destruction with exposed subchondral bone	Full thickness defect(s)	

Table 1. Outerbridge classification of cartilage defect.

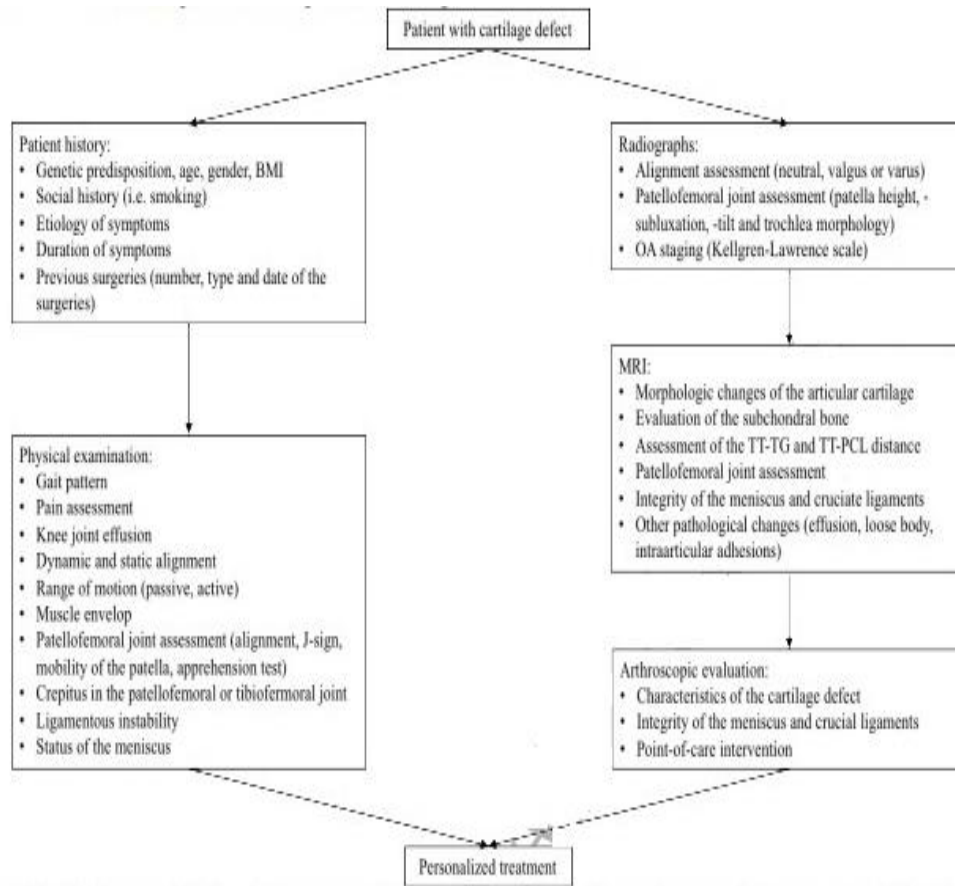


Fig. 5. Cartilage defect diagnostic plan

TREATMENT OPTIONS FOR CARTILAGE INJURY

CONSERVATIVE TREATMENT

SURGICAL TREATMENT: Arthroscopic lavage and debridement, Abrasion arthroplasty, Subchondral drilling, Microfracture, Bone marrow stimulating procedures. Osteochondral Grafting, Osteochondral Allograft Transplantation, Autologous Chondrocyte Implantation, Matrix-induced ACI (MACI)

THE SPECIAL PART

3. SURGICAL PROCEDURE WITH HISTOLOGY ANALYSIS AND RESULTS

The present research study aimed to assess magnetic resonance imaging (MRI) changes and histological findings in the therapeutic effects of microfractures in the treatment of complex animal knee lesions resulting from osteochondral and meniscal defects resulting from non-total meniscectomies.

One of the endpoints of this study was to evaluate the efficacy of microfracture technique in surgically-induced cartilage and subchondral bone lesions along with meniscal in the rabbit knee.

Another endpoint was to analyze histological modifications after lesions were created.

The third end point was to analyze the MRI images of the healing process.

The motivation of this research was to standardize the procedure for MRI examination of the articular surface of the knee rabbit used in this study.

The study protocol was crucial to the current thesis' experiment since it served as the primary tool for assessing how well the articular surface had been restored. With the development of new conservation technologies, surgical techniques, and the variety of grafts used in the profile industry, surgical procedures for the restoration of articular cartilage that aim to preserve the knee joint or delay the need for knee replacement surgery are currently being studied extensively.

The findings of this study provide valuable insights into the use of microfractures in the treatment of knee cartilage repair tissue. By using a rabbit model and evaluating the repair tissue through MRI, this study highlights the potential of microfracture treatment to produce hyaline-like cartilage with good mechanical properties.

Although knee arthroplasty is also regarded as a bone stock-saving operation, the surgical reason for replacing the joint with an endoprosthesis is no longer accepted due to the revision rate's rise in recent years and the hazards associated with the intra- and postoperative phases of the procedure.

3.2. MATERIALS AND METHODS

The experimental model was built in order to reproduce in an experimental context a situation encountered relatively frequently in usual practice, namely, the lesional association at the level of the knee consisting of the duo meniscal lesion - osteochondral lesion.

Twenty-two male adult rabbits, belonging to the New Zealand White breed were selected for the present study. The rabbits were purchased from the Cantacuzino Institute and acclimatized in the biobase of the University of Agricultural Sciences and Veterinary Medicine of Banat Timișoara where the experimental part was carried out with the approval of the University's Bioethics Committee and the approval of the Veterinary Sanitary Directorate and CECS approval of U.M.F.T.

The rabbit was chosen because it presents the biomechanical advantage of total loading of the knee during normal walking, replicating human joint stress where the loading is also produced with full weight bearing. All rabbits were adults, older than three years old, and were selected to replicate the biological context of the adult and avoid false results due to the biological reserve of youth.

In order to exclude possible result errors due to the involuntary influence of the researchers, the animals were divided into experimental groups after the initial operations (baseline) to ensure the uniformity of the created lesions and not to involuntarily influence the data depending on the created lesions.

After the initial surgery all animals were marked with a subcutaneous CIP with a unique code to avoid errors after allocation to study groups and for ease of recognition at the end of the follow-up period.

3.2.1. Surgical procedure

The first surgical procedure consisted of creating the full thickness osteochondral defect on the weight bearing surface of the internal femoral condyle, and partial meniscectomy of the internal meniscus, under general anesthesia using Ketamine (35 mg/kg) and Xylazine (5 mg/kg).

The integrity of the articular cartilage was assessed by open arthrotomy. The site of choice for performing the osteochondral lesion was the internal femoral condyle, the motivation also being the most faithful simulation of the situations encountered in medical practice in which the internal compartment of the knee is most often affected, especially the internal femoral condyle.

The osteochondral injury was created with the scalpel at the level of the load-bearing surface of the internal femoral condyle in the entire thickness of the cartilage with the exposure of the subchondral bone (Fig. 6 and Fig. 7).

At the end of the intervention, the surgical wound was sutured in anatomical planes and wound dressing with mean duration of surgery of 16 minutes and post-operative observation for six hours. The experimental animals were properly cared for with analgesia and antibiotic prophylaxis according to the regulations of the biobase. As immediate complications, there were two cases with superficial infection of the operative wound, but with favorable evolution under treatment and care.

After the induced defect, the twenty-two rabbits were separated in two equal groups consisting of 11 rabbits:

- **the control group (C1):** the group of animals that were not therapeutically intervened in any way. After the healing of the operative wound, the osteochondral lesion and the meniscus lesion were allowed to evolve naturally, the animals had no movement or feeding restrictions.
- **the microfractures group (MF2):** after the initial surgery, the MF2 group underwent a second surgical procedure that consisted in producing microfracture like osteochondral tunnels using a 2.0 mm Kirschner wire (K wire).

The post-operative ("post-traumatic") follow-up period was 6 months after the initial intervention to ensure the onset and evolution of degenerative changes in the three experimental groups.

3.3.2. Results

Of the ten cases included in our study, we observed normal histological aspects on morphological staining with haematoxylin-eosin in one case (Figure 8). In five cases we observed repair processes after microfractures. Only one case of those included in the study showed an inflammatory process, in this case we noted morphological changes of septic arthritis.

The healthy knee has articular cartilage and adjacent bone tissue in its structure. Articular cartilage is of the hyaline type consisting of chondrocytes arranged in isogenic groups surrounded by homogeneous, acidophilic extracellular matrix. Beneath the cartilage is an area of compact bone tissue, which differs from cartilage in the presence of osteons formed by concentric bone lamellae arranged around a canal. The bone lamellae have osteocytes arranged in isolation in

gaps in the bone matrix. The irregularly shaped spaces present in bone tissue represent resorption canals containing osteoblasts, osteoclasts and blood vessels.

Their presence indicates an active bone remodelling process. Following the compact bone is cancellous bone made up of irregularly shaped bony trabeculae. The spaces within cancellous bone contain bone marrow consisting mainly of unilocular white adipocytes and small islands of myeloid tissue.

In the five cases with microfractures, a repair process is observed following the lesion, involving endochondral and membrane ossification, as well as involvement of the periosteum (the outer connective sheath of the bone) and adjacent soft tissues.

Cases with microfractures showed areas containing hypertrophic chondrocytes in lacunae, but most chondrocytes in all sections were small.

In one case we observed morphological changes of septic arthritis. The synovial membrane is oedematous, and the joint cavity contains inflammatory exudate rich in leukocytes.

Microscopic examination of the reparative tissue was characterized by the presence of fibrocartilaginous tissue. Collagen fibres are oriented tangentially to the cartilage. Under the fibrocartilaginous tissue, hyaline cartilage-like tissue and then hyaline cartilage with small chondrocytes arranged in isogenic groups and homogeneous, acidophilic extracellular matrix are observed. Hyaline cartilage showed some degenerative changes and cracking (Figure 14).

In three cases included in the study, the presence of hyaline articular cartilage is noted, under which immature bone tissue is present, and mature bone is observed in its continuation.

3.4. IMAGING ANALYSIS STANDARDIZATION OF ARTICULAR CARTILAGE IN CARTILAGE LESSIONS

Six months after the initial procedure both groups were assessed using imagistic investigation. MRI was performed to detect the evolution of the induced defect and the subchondral oedema for each rabbit. For the MRI imaging examination, we used a Siemens Magnetom Essenza 1.5 Tesla unit. MRI exam was done according to knee protocol: sagittal plane scans in T1-weighted, T2-weighted, proton density (PD) and turbo spin echo (TSE), coronal plane scans T1, turbo inversion recovery magnitude (TIRM), PD, TSE with or without fat-saturation and transverse scans PD, TSE with fat-saturation.

4. DISCUSSIONS

The microfracture technique was introduced in the early 1980s by Steadman and colleagues, being described as a treatment for full-thickness posttraumatic cartilage defects. It is a marrow stimulation technique and as opposed to other similar techniques, such as drilling, there is essentially no risk for thermal necrosis of surrounding tissue. Concerning the drilling technique, Menche and co-workers proved that in Pridie drilling, the repair tissue lasted longer than in abrasion chondroplasty. The authors carried out a study over thirty-nine rabbits in which they performed the Pridie drilling intervention in the left knee and abrasion chondroplasty in the right knee. Microfractures prove to be a valid therapeutic option in dealing with an association of knee pathologies, such as small chondral defects and meniscal tears. This procedure consists of marrow stimulation by arthroscopic penetration of the subchondral plate using a key wire. Creating tunnels in the subchondral plate determines subsequent bleeding, enabling the inflow into the defect of blood, blood-derived cells, and bone marrow-derived mesenchymal stem and progenitor cells (MSCs). The blood clot that forms contains growth factors, platelets, and bone marrow-derived pluripotent stem cells which determine fibrocartilaginous repair tissue. Although microfracture is a good first-line option for cartilage defect surgery, being a simple technique and effective procedure, it is preferred to be used for small defects, in young patients.

There are several studies related to the effectiveness of microfractures in the knee joint on the early, mid- and long-term assessment. Microfracture represents a key first-line treatment option for small articular cartilage defects of the knee joint and provides good function and pain relief at the mid-term and satisfying results thereafter. In an editorial, Song and co-workers raised questions regarding suboptimal repair with fibrocartilage infill, subchondral osseous overgrowth, questionable durability of fibrous cartilage, and deterioration of clinical improvement over long-term follow-up.

When treating a group of knee disorders, including chondral defect and meniscal tear, microfractures demonstrate to be a viable therapeutic approach. The technique known as microfracture involves arthroscopically penetrating the subchondral plate with a keywire to stimulate the marrow. Making tunnels in the subchondral plate causes more bleeding, allowing blood, blood-derived cells, and bone marrow-derived mesenchymal stem and progenitor cells (MSCs) to enter the defect. Growth factors, platelets, and pluripotent stem cells generated from

bone marrow are present in the blood clot that forms and determine the fibrocartilaginous repair tissue. Being a straightforward technique and efficient operation, microfracture is a good first-line alternative for treating cartilage defects, however it is preferable to be utilized for tiny lesions in young patients.

Therefore, this experimental study has assessed the therapeutic benefits of microfractures in the treatment of this complex pathology using MRI seriated examinations, proposing at the same time an algorithm of multistage MRI imaging examination. In our opinion, the multistage MRI examination can correlate also post-operative recommendations and weight-bearing indications for different healing stages, according to the image characteristics on MRI, which can prove useful in a rehabilitation protocol following this type of surgical repair for osteochondral defects.

5. CONCLUSIONS

The results of this study offer important new understandings regarding the usage of microfractures in the therapy of knee cartilage repair tissue. This study demonstrates the possibility of microfracture treatment to produce hyaline-like cartilage with good mechanical properties by employing a rabbit model and assessing the repair tissue by Magnetic Resonance Imaging. The results also point to Magnetic Resonance Imaging as a potentially useful tool for gauging the success of microfracture therapy and tracking the healing process.

We could also validate the results of microfracture treatment technique from the correlation of histology findings and MRI images.

The findings of the investigation could influence future research in this field and have significant ramifications for the creation of successful treatments for cartilage damage. This work emphasizes the significance of continuous research into new methods for cartilage healing and the potential of magnetic resonance imaging to assist in these efforts.

The objectives proposed in the doctoral research where, as follows:

- to confirm the regenerative potential of the microfracture treatment at the level of the induced cartilage defect, respectively the evaluation of the treatment result using non-invasive imaging methods, with the simulation in experimental conditions of a situation frequently encountered in the clinic and without satisfactory solutions at the present time;
- to validate the prophylactic role of this therapy in the evolution and progression of chondropathy towards gonarthrosis at a relatively young age;
- to present a comparative study of the results between the patients and the control group, using imaging means, respectively MRI;
- to evaluate the effect of microfracture therapy in the management of an extremely difficult pathology to address, namely lesions on the articular surface non-compliant with conservative treatment measures;
- to propose the MRI examination as an extremely efficient technique in the evaluation of the joint surface in order to evaluate the remodeling capacity of these therapies on the cartilage, were fulfilled following the study described in the thesis.

The following conclusions are reached as a result of the research work done for the PhD studies:

- Microfracture therapy accelerates the physiological healing process and improves the final result of the remodeling of the articular surface by limiting the global degeneration of the joint after trauma, resulting in cartilage lesion complex in an animal model. The protocol of regenerative restoration of the damaged articular surface was used for the microfracture technique in the subchondral socket, and the mobilization of the post-procedure joint was not limited in any way.
- The results regarding the articular cartilage regeneration therapy through microfractures in the subchondral bone are scientifically valid for two major reasons: post-therapy, the articular surface with the induced defect showed traces of bleeding at the level where the treatment was applied, which proves to us that a path for the migration of mesenchymal cells and growth factors; respectively, the results after the imaging examination show the improvement of the condition of the articular cartilage compared to the control group.
- Microfracture therapy can bring a major benefit in the pathology of cartilage lesions, both focal and degenerative, that do not respond to other options of conservative treatment, taking into account a multitude of factors that can influence the final result in one way or another, such as be: the age of the patient, the area of the cartilage lesion, the surgical technique.

Personal contributions:

- Replication in experimental conditions on an animal model of a relatively frequent cynical situation at the level of the knee, with a lesional complex consisting of a post-traumatic cartilage defect diagnosed at the time of surgery for various pathologies at the level of the knee joint.
- Histologic analysis of the specimen and highlighting different stages of the repairing process and formation of fibrocartilaginous tissue.
- The use of MRI examination for the detailed description of the articular surface defect and the biomechanical involvement in the arthrosis process of the contact surface disorders.
- Standardization of the osteochondral fragment analysis technique using MRI examination.
- The proposal of a minimally invasive surgical option for the treatment of articular cartilage lesions at the level of the knee joint in order to preserve the joint and postpone the moment of its replacement by arthroplasty in the young adult.