Original Article

Replacement of osteochondral defect of large joints: An experimental model in sheep

Georgii Alexandrovich Airapetov^{*,1}, Oshozimhede Emeghomhe Iyalomhe, Faeren Matilda Adzege

Department of Traumatology and Orthopedics, Stavropol State Medical University, Russian Federation

A B S T R A C T

Aim: To improve the treatment results of patients with osteochondral defects of large joints.

Materials and methods: A prospective study was conducted on 30 animals (60 knee joints) aged 1.5–3 years. All animals were divided into 3 groups of 10 each, depending on the method of replacement of the osteo-chondral defect.

Results: 6 months after intervention using the proposed method, a good prognosis can be given and good long-term results expected despite the full axial load on the joint a few hours after the operation.

1. Introduction

Undoubtedly, injuries and diseases of large joints and effective long-term methods of treatment and management remains an orthopedic problem. More than 50% of visits to an orthopedic doctor in an outpatient unit are associated with degenerative - dystrophic diseases^{1,2} and 60% of these patients have chondromalacia of varying degrees.³ There are various methods of treating such pathology. The main principles of conservative treatment involve pain relief, elimination of causes contributing to the progression of the disease and restoration of lost functions. However, the effectiveness of such treatment remains doubtful.^{4–6} Unlike conservative methods of treatment, new and advanced surgical methods are being continuously developed.^{7–10}

One of the first methods of treating osteochondral defects, proposed in the 1960s is tunneling. This method continues to be used in practice.¹¹ A disadvantage of tunneling is burning of nearby tissues due to incorrect drilling, leading to reduced bleeding from the subchondral bone and prevention of formation of a full-fledged and stable chondroid fibrous tissue.¹² Another common and fairly effective method of treatment of osteochondral defects, proposed by Steadman in 1997, is microfracturing. Despite all the advantages, there is doubt about the need to perform microfracturing, as the chondroid fibrous tissue formed is unable to withstand stress and rapidly undergoes lysis.¹³

1.1. Aim of the research

To improve the treatment results of patients with osteochondral

defects of large joints.

2. Materials and Methods

A prospective study was conducted on 30 animals (60 knee joints) aged 1.5–3 years. Ewes were used in the model due to the anatomical similarity of the knee joint with the human knee joint, hence, making it possible to make an analogy. They have high tolerance for anesthesia, and can be used as experimental animals on the territory of the Russian Federation (Law “On the Protection of Animals Against Ill-Treatment”, dated December 1, 1999, Art. 9, clause 4). All experiments were in compliance with the ARRIVE guidelines.

All animals were divided into 3 groups of 10 each, depending on the method of replacement of the osteochondral defect. In all joints, a full-thickness defect of 4.5 mm with a subchondral bone was formed in the region of the medial condyle of the femur. One of the joints where the formed defect was not replaced served as control for the experiment. In group 1, animals were treated by microfracturing after the defect had been formed. In group 2, the defect was replaced by extracellular collagen matrix. In group 3, extracellular collagen matrix stitched to the edges of the defect using absorbable suture thread, platelet-rich plasma (PRP) was injected under the matrix and shredded hyaline cartilage from the non-loaded joint surface was used. The cartilage was crushed to 1 mm and injected under the membrane at the rate of 5 fragments per cm² of defect. The wound was sutured in layers. As an anesthetic, 2% Rometarii solution was used.

The animal was placed on its side, shaved, after which the operating

^{*} Corresponding author.

E-mail address: airapetovga@yandex.ru (G.A. Airapetov).

¹ Substitution of knee bone cartilage defects.

Fig. 1. 4.5 mm full-layer defect on the loaded surface of the sheep's knee joint.

Fig. 2. Hemmed extracellular collagen matrix to defect.

field was treated with antiseptic. The limb was placed in a flexed position and a 4 cm surgical incision was made at the site of projection of the patellar ligament. The next stage was the incision of subcutaneous fat and joint capsule to obtain access to the medial condyle of the knee joint. A cutter with a diameter of 4.5 mm was used to perform osteochondral intersection (Fig. 1.). The next step was to replace the formed defect using different methods. This was dependent on the experimental group (Fig. 2). The wound was sutured in layers, without leaving a drainage in the postoperative wound.

All animals were kept in conditions in accordance with the requirements of the legislation of the Russian Federation. Load on the limb was allowed immediately after the operation.

The site of operation and newly formed tissue was studied visually and morphologically using light microscopy. For morphological studies, the material was fixed using 10% formalin and dehydrated. This article presents the results 6 months after surgery.

3. Results of the study

All animals underwent a period of observation without any remarkable changes. Infectious complications were not observed in any group.

6 months after the operation, in group 1 where microfracturing was performed, tissue defect was observed, the bottom of the osteochondral defect was eroded, and the boundary between the defect zone and its own hyaline cartilage was clearly visible (Fig. 3).

The microscopic view of the area of the osteochondral defect is shown in Fig. 4. 6 months after the formation of the defect, there was tissue defect about 1/2 of the thickness of the formed defect with fine smooth edges. The boundary between the hyaline articular cartilage and newly formed tissue could be clearly traced, as it was sanguineous.

Fig. 3. Macroscopic picture in group 1.

Fig. 4. Fragment of the medial femoral condyle of a ewe. Full layer defect of the articular hyaline cartilage and sub-chondral bone plate, hematoxylin-eosin staining, magnification x 40.

Microscopically, a thin, discontinuous layer of newly formed chondroid tissue was seen at the bottom of the articular cartilage defect. The osteochondral defect consisted of coarse-fibrous connective tissue with a large number of thick-walled vessels filled with non-lysed erythrocytes and collagen structures were fibrous.

6 months after surgery in group 2, where the defect was replaced using extracellular collagen matrix, tissue defect which was 2/3 the thickness of the previously formed osteochondral defect was observed. The edges of the osteochondral defect were even, absence of eroded surfaces and the boundary between newly formed tissue and hyaline cartilage was not clearly traced (Fig. 5).

Microscopically, in the group where the defect was replaced using extracellular collagen matrix, a more stable layer was observed. There were signs of cartilaginous tissue remodeling, expressed in the formation of column-pillars by chondrocytes, there was some surface layer recovery indicating increase in cell number and appearance of isogenic group of cartilaginous cells in the matrix. These changes indicate a partial restoration of the cartilage structure, which however, has not reached the state of mature tissue cartilage (Fig. 6).

6 months after surgery in group 3, where the defect was replaced using an extracellular collagen matrix, PRP and crushed auto-cartilage, the defect was completely replaced. The replaced defect had smooth even edges, the border between newly formed tissue and the preserved

Fig. 5. A macroscopic picture of Group 2.

Fig. 6. Fragment of the medial condyle of the femur of a ewe. Full-layer defect of the articular hyaline cartilage and subchondral bone plate, stained with hematoxylin-eosin, magnification x 200.

Fig. 7. Macroscopic picture group 3.

Fig. 8. Fragment of the medial condyle of the femur of a ewe. Full-layer defect of the hyaline cartilage and subchondral bone plate, hematoxylin-eosin staining, magnification x 100.

articular hyaline cartilage was poorly traced (Fig. 7).

6 months after the start of the experiment, on histological sections, at the site of damage to hyaline cartilage, a heterogeneous layer of chondroid tissue 4/5 the thickness of the adjacent intact cartilage was formed, leaving a slight depression with a rather uneven base.

In the thickness of hyaline-like cartilage, a hypocellular matrix containing lacunae with mature chondrocytes, 2–5 cells each was observed. At the same time, in part of the field of view, in the deep and middle regions of the newly formed cartilage, the vertical columnar of the lacunae was traced (Fig. 8), while in the surface regions, separate lacunae with isolated cells among moderate amount of the intercellular matrix was observed. On the surface of the formed cartilage and fibrous connective tissue, there was formation of a fibrous layer, similar to a forming perichondrium.

Subchondral plate was completely restored with the exception of the central part of the formed defect; its fibres were thick, formless, mostly perpendicular, interstitial substance was moderately homogenous.

4. Discussion

Most experts believe that osteochondral defects require surgical treatment.¹⁴ These defects are mainly diagnosed using MRI, which improves preoperative evaluation and contributes to surgical results. One of the popular methods of treating such defects is microfracturing. This is a simple and cheap technique performed arthroscopically. According to many authors, this technique makes it possible to obtain good results in majority of clinical cases.^{15–18} However it has been suggested that the newly formed tissue does not fully replace or plug-in the defect and quickly undergoes lysis. This was demonstrated by the results obtained in group 1. Another popular method of replacing such defects is mosaic autochondroplasty. Despite positive results in using this method, there is high probability of developing pain in the donor area, and the chance of development of degenerative-dystrophic diseases in such a joint may be higher.¹⁹

Methods for chondrocyte culture and implantation on the matrix or under the material separating the defect area from the joint cavity are actively being developed. Autologous Chondrocyte Implantation (ACI) and Autologous Matrix Induced Chondrogenesis (AMIC) are examples of these methods. Both methods show good results, however, in ACI, 2 surgeries need to be performed, and AMIC has a high cost.²⁰

There is active research into the use of mesenchymal stem cells, however, these methods have several drawbacks.^{21,22}

In this research, an attempt was made to combine the advantages of using monotherapeutic agents such as extracellular collagen matrix,

PRP and autoplasty. The proposed method can be performed during a single, in contrast to the ACI method, is less aggressive than mosaic autochondroplasty, and does not leave large defects in the donor area.

The results obtained in the group where microfracturing of the defect was performed show the inferiority of the formed clot and its early lysis. The most optimistic results are observed in group 3, where there was formation of a heterogeneous layer of chondroid tissue 4/5 the thickness of the adjacent intact cartilage. Morphologically, it was possible to trace the structure of the chondroid tissue, which, in our opinion, may indicate good results.

5. Conclusion

6 months after intervention using the proposed method, a good prognosis can be given and good long-term results are expected despite the full axial load on the joint a few hours after the operation. This method allows a relatively complete replacement of the defect area with newly formed tissue characteristic of normal hyaline cartilage. The next stage in this research is approbation and induction of the proposed method in clinical practice.

Competing interest

The authors have no competing interests to declare.

References

- Marlovits S, Striessnig G, Resinger CT, Aldrian SM, Vecsei V, Imhof H, Trattnig S. Definition of pertinent parameters for the evaluation of articular cartilage repair tissue with high-resolution magnetic resonance imaging. *Eur J Radiol*. 2004;52(3):310–331.
- Widuchowski W, Widuchowski J, Trzaska T. Articular cartilage defects: study of 25,124 knee arthroscopies. *Knee*. 2007;14:177–182.
- Flanigan DC, Harris JD, Trinh TQ, et al. Prevalence of chondral defects in athletes' knees: a systematic review. *Med Sci Sports Exerc*. 2010;42:1795–1801.
- Cain EL, Clancy WG. Treatment algorithm for osteochondral injuries of the knee. *Clin Sports Med*. 2001;20:321–342.
- Andia I, Abate M. Knee osteoarthritis: hyaluronic acid, platelet-rich plasma or both in association? *Expert Opin Biol Ther*. 2014;14(5):635–649.
- Chang KV, Hung CY, Aliwarga F, Wang TG, Han DS, Chen WS. Comparative effectiveness of platelet-rich plasma injections for treating knee joint cartilage degenerative pathology: a systematic review and meta-analysis. *Arch Phys Med Rehabil*. 2014;95(3):562–575.
- Dhollander A, Moens K, Van der Maas J, Verdonk P, Almqvist KF, Victor J. Treatment of patellofemoral cartilage defects in the knee by autologous matrix-induced Chondrogenesis (AMIC). *Acta Orthop Belg*. 2014;80:251–259.
- Gomoll AH, Farr J, Gilligly SD, et al. Surgical management of articular cartilage defects of the knee. *Instr Course Lect*. 2011;60:461–483.
- Farr J, Cole B, Dhawan A, Kercher J, Sherman S. Clinical cartilage restoration: evolution and overview. *Clin Orthop Relat Res*. 2011;469:2696–2705.
- Ulstein S, Aroen A, Røtterud JH. Microfracture technique versus osteochondral autologous transplantation mosaicplasty in patients with articular chondral lesions of the knee: a prospective randomized trial with long-term follow-up. *Knee Surg Sports Traumatol Arthrosc*. 2014;210–216.
- Pridie K. A method of resurfacing osteoarthritic knee joints. *J Bone Joint Surg Am*. 1959;41:618–619.
- de Windt TS, Bekkers JE, Creemers LB, et al. Patient profiling in cartilage regeneration: prognostic factors determining success of treatment for cartilage defects. *Am J Sports Med*. 2009;37(suppl 1):58S–62S.
- Mithoefer K, Williams 3rd RJ, Warren RF, et al. The microfracture technique for the treatment of articular cartilage lesions in the knee. A prospective cohort study. *J Bone Joint Surg Am*. 2005;87:1911–1920.
- Wang D, Jones MH, Khair MM, Miniaci A. Patient-reported outcome measures for the knee. *J Knee Surg*. 2010;23:137–151.
- Burr DB, Radin EL. Microfractures and microcracks in subchondral bone: are they relevant to osteoarthritis? *Rheum Dis Clin N Am*. 2003;29:675–685.
- Kreuz PC, Erggelet C, Steinwachs MR, et al. Is microfracture of chondral defects in the knee associated with different results in patients aged 40 years or younger? *Arthroscopy*. 2006;22:1180–1186.
- Ulstein S, Aroen A, Røtterud JH, Løken S, Engebretsen L, Heir S. Microfracture technique versus osteochondral autologous transplantation mosaicplasty in patients with articular chondral lesions of the knee: a prospective randomized trial with long-term follow-up. *Knee Surg Sports Traumatol Arthrosc*. 2014;38–42.
- Knutsen G, Engebretsen L, Ludvigsen TC, et al. Autologous chondrocyte implantation compared with microfracture in the knee: a randomized trial. *J Bone Joint Surg Am*. 2004;86:455–464.
- Krych AJ, Harnly HW, Rodeo SA, et al. Activity levels are higher after osteochondral autograft transfer mosaicplasty than after microfracture for articular cartilage defects of the knee: a retrospective comparative study. *J Bone Joint Surg Am*. 2012;94:971–978.
- McNickle AG, L'Heureux DR, Yanke AB, et al. Outcomes of autologous chondrocyte implantation in a diverse patient population. *Am J Sports Med*. 2009;37:1344–1350.
- Mafi P, Hindocha S, Mafi R, Griffin M, Khan WS. Sources of adult mesenchymal stem cells applicable for musculoskeletal applications- a systematic review of the literature. *Open Orthop J*. 2011;5:238–244.
- Zhai LJ, Zhao KQ, Wang ZQ, Feng Y, Xing SC. Mesenchymal stem cells display different gene expression profiles compared to hyaline and elastic chondrocytes. *Int J Clin Exp Med*. 2015;4(1):81–90 P. 5–11.