Supplementary Materials for:

Investigation on decellularized live hyaline cartilage grafts material for efficient treatment of knee cartilage defects

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S1 Clinical data and methods

S1.1 General Information

Inclusion criteria:

(1) Subjects diagnosed with acute or chronic knee cartilage injury through clinical physical examination and MRI examination;

(2) Subjects aged between 18 and 65 years old;

(3) Subjects who meet the ICRS Grade III-IV criteria;

(4) Subjects with knee cartilage injury area of 2-4cm2;

(5) Subjects with unilateral knee cartilage injury requiring surgical treatment;

(6) Participants who voluntarily sign a written informed consent form and are able to complete the prescribed follow-up according to the requirements of the clinical protocol;

(7) The researchers believe that the participants are suitable for participating in this clinical trial.

Exclusion criteria:

(1) Subjects with a body mass index (BMI) greater than 30 kg/m2;

(2) The knee joint space is significantly narrowed;

(3) Subjects who receive long-term hormone therapy or intra-articular injection of hormones;

(4) Subjects with severe subchondral bone injury and ipsilateral severe meniscus injury on MRI, requiring complete meniscectomy;

(5) Knee cartilage injury is caused by infections, malignant tumors, rheumatism, and autoimmune diseases (rheumatoid arthritis, lupus erythematosus, etc.) in subjects;

(6) Subjects with systemic infections, including HIV, hepatitis C, and syphilis;

(7) The source of cartilage repair material is pig derived chondrocytes, and subjects with special beliefs.

A total of 27 patients with knee cartilage injuries were included and treated with arthroscopic decellularized cartilage repair material (DLHCG) repair surgery. All patients signed informed consent forms. This study was approved by the Medical Ethics Committee of Yantai Mountain Hospital in Yantai City (LL-2022-046-K).

S1.2 Surgical methods

After spinal anesthesia, the patient is placed in a supine position with the knee joint flexed. Routine disinfection, placement, and use of pneumatic tourniquets to stop bleeding in the affected limb.

First, perform a knee arthroscopy examination to determine the damaged area of the knee joint cartilage. The damaged cartilage of the knee joint is then repaired, shaped, and rejuvenated. The area of the damaged cartilage is measured using a probe hook. The cartilage repair material is then stretched and cut using sterile aluminum foil paper. After suctioning out the joint cavity fluid, the cartilage repair material is accurately placed in the knee joint cartilage defect area using instruments. The probe hook is used to spread and translate the graft, and it can be seen that the graft is completely attached. Protein adhesive is injected. No indwelling drainage, suture the incision, and apply pressure bandaging.

S1.3 Postoperative Management and Rehabilitation

Following the principle of early activity and late weight-bearing, for patients with tibiofemoral joint cartilage injury, postoperative straight position fixation with range of motion: 30 ° at 3 weeks, 60 ° at 6 weeks, 90 ° at 8 weeks, and 130 ° at 12 weeks. Avoid weight-bearing for 6 weeks after surgery, partially load during 6-12 weeks, and fully load after 12 weeks. Fix the support for 12 weeks.

Resume normal life and low-intensity physical activity 6 months after surgery.

S1.4 Observation indicators

MOCART score was used to evaluate the bone and cartilage healing in the cartilage defect area before surgery, 3 months after surgery, 6 months after surgery, and 12 months after surgery. Visual Analog Scale (VAS) was used to assess pain. Lysholm score, Tegner score, and subjective IKDC score were used to evaluate the subjective symptoms, objective signs, and knee joint function of the knee joint system.

S1.5 Statistical analysis

Using SPSS 22.0 statistical software, the Shapiro Wilk method was used to perform normality tests on the data. Metric data that conforms to a normal or near normal distribution are represented by X ± s.

S2. Discussion

If knee cartilage injury is not intervened, it may eventually develop into knee osteoarthritis and other diseases, requiring unicompartmental or total knee arthroplasty. Therefore, early intervention in cartilage injury treatment can not only repair the damaged cartilage, but also avoid joint replacement surgery. There are many methods for repairing and treating knee cartilage injuries in clinical practice, including microfracture technology, osteochondral cell transplantation technology, autologous chondrocyte transplantation technology, matrix induced autologous chondrocyte transplantation technology, tissue engineering, and stem cell cartilage repair technology. Dhillon et al. [1] found that the failure rate of microfracture treatment was 2.5% -8.3%, and the failure rate of autologous chondrocyte transplantation treatment was 0% -1.8%. In addition, the postoperative Lysholm score, Tegner score, and subjective IKDC score of the autologous chondrocyte transplantation group were significantly improved. Although we can use biologics such as platelet rich plasma, hyaluronic acid, mesenchymal stem cells, and concentrated bone marrow blood to enhance microfracture technology, which can improve Lysholm score, IKDC score, and MOCART score, fibrocartilage is still formed, not hyaline cartilage [2-4]. The osteochondral transplantation technique (OATS technique) can temporarily restore the damaged cartilage surface, but due to postoperative weight-bearing causing the graft to sink and a lack of repair in the dead space between cylindrical grafts, this may lead to eventual repair failure, and the donor source for autologous cartilage transplantation is limited. Autologous chondrocyte transplantation (ACI) is suitable for young patients with large cartilage defects and is currently the gold standard for cartilage repair [5]. Due to the need for in vitro expansion, secondary surgery is necessary, and the loss of chondrocytes during treatment can seriously affect the final repair treatment due to the nature of the implant fluid. Cell free scaffold technology is suitable for small cartilage defects. Cell free scaffolds induce the migration of surrounding normal autologous chondrocytes into the scaffold to complete cartilage repair. Studies have pointed out the problem of excessive bone growth, and their cartilage repair effect is worse than that of cell transplantation materials [6-8]. The cartilage repair material (DLHCG) used in this study combines the advantages of cartilage tissue engineering and decellularization technology. It is a decellularized allogeneic transparent cartilage graft, with the main components being similar to articular cartilage. It can induce in situ regeneration of high-purity transparent cartilage phenotype articular cartilage. This cartilage repair material using extracellular matrix can better allow cells in the tissue to adhere, proliferate, and differentiate [9]. In the preliminary animal experiments, the repair effect of cartilage repair materials was good [10]. The results of this study indicate that arthroscopic DLHCG can effectively repair knee cartilage injuries

without adverse reactions, and the preliminary results are good.

S2.1 Advantages and disadvantages of cartilage repair materials (DLHCG)

The advantages of using cartilage repair material (DLHCG) to treat knee cartilage defects mainly include:

(1) Expand the sources of tissue transplants. The decellularization method used in decellularized allogeneic transparent cartilage grafts expands the sources of tissue grafts, allowing for selection from autologous, allogeneic, and heterologous sources, as the main source of immunogens - cellular components - has been eliminated [11].

(2) Promote seamless connection and integration of transplants. This cartilage repair material combines the advantages of cartilage tissue engineering and decellularization technology, allowing host chondrocytes to be transplanted to the transplantation interface, enabling new cartilage tissue to develop in situ in cartilage defects, promoting seamless connection and integration of transplantation, restoring the full thickness of defective cartilage, and forming a common, continuous, and smooth cartilage surface [12]. LhCG (living transparent cartilage graft) has relatively pure transparent cartilage tissue and phenotype. As a direct decellularization product of LhCG, dLhCG inherits the composition and properties of transparent cartilage [10].

(3) Avoiding secondary surgery results in good recovery of knee cartilage and function. This cartilage repair material surgery can be completed in one stage, without the need for cell collection and culture, nor the need to collect natural cartilage for transplantation, avoiding secondary surgical trauma for patients and reducing economic burden. In the results of this study, the MOCART score, VAS score, Lysholm score, Tegner score, and subjective IKDC score of patients at 3 months, 6 months, and the last follow-up were significantly improved compared to preoperative levels, indicating that the patient's cartilage was repaired and knee joint function was also improved.

However, there are also shortcomings in the application of this cartilage repair material: (1) the thickness of the cartilage defect area needs to be measured, otherwise, if the graft is too thin, it is prone to damage and wrinkles during implantation. If the graft is too thick, it will be higher than the normal joint surface after implantation, which is prone to wear and tear after surgery, affecting the effectiveness of cartilage repair; (2) The transplant is fixed with protein adhesive, which is not as precise as suture fixation. The postoperative joint movement and weight-bearing time are relatively long.

S2.2 Key operating points of cartilage repair material (DLHCG)

1. Formation and Freshness of Cartilage Defects: It is necessary to repair and shape the damaged cartilage area, and make the area fresh; 2. Measurement of cartilage defect area: After rough measurement of the cartilage transplantation area using equipment such as hooks and plasma, the size of the defect area is detected. It is important to note that the graft should not be larger than the cartilage defect area to avoid the graft being unable to be inserted; 3. Transplant preparation: Based on the size of the cartilage defect area, first expand the sterile aluminum foil paper, and then place the cartilage repair material on the expanded sterile aluminum foil paper for precise cutting; 4. Preparation for graft implantation: Lay the graft flat on a specialized delivery device, gently and slowly, to avoid causing the graft to break or wrinkle; 5. Spread and fix the graft: Use a probe hook to spread and translate the graft, allowing it to fully adhere to the cartilage defect area, and inject protein adhesive. We can also use bone marrow aspiration concentrate (cBMA) to enhance cartilage repair, which helps stimulate tissue response for transparent cartilage repair, but the specific treatment method is currently unclear [13, 14].

S2.3 Complications and Prevention of Cartilage Repair Materials (DLHCG)

Although DLHCG has undergone decellularization treatment, there are still residual non autologous ECM and trace amounts of non-autologous cell residues, which may pose a potential risk of allogeneic or allogeneic transplant rejection. However, because cartilage tissue does not have vascular properties, and animal experiments have not caused transplant rejection reactions in highly vascularized omentum, it can be safely used for joint cartilage injury repair. The application of decellularized scaffolds in cartilage defect repair lacks long-term clinical evidence support [13].

The main shortcomings of this study are as follows: firstly, there are few enrolled patients and a small sample size; Secondly, the follow-up time is short, and related complications may not have been manifested yet. In response to these two points, this study will continue to increase the sample size and extend the follow-up time. In addition, we will continue to pay attention to the research on the treatment of knee cartilage defects, and achieve a combination of horizontal and vertical analysis.

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