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The detached osteochondral fragment as a source of cells for autologous chondrocyte implantation (ACI) in the ankle joint

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Summary

Objective: Autologous chondrocyte implantation (ACI) has been successfully used for the treatment of osteochondral lesions of the talus. One of the main problems of this surgical strategy is related to the harvesting of the cartilage slice from a healthy knee. The aim of this study was to examine the capacity of chondrocytes harvested from a detached osteochondral fragment to proliferate and to serve as a source of viable cells for ACI in the repair of ankle cartilage defects.

Methods: Detached osteochondral fragments harvested from the ankle joint of 20 patients with osteochondral lesions of the talus served as the source of human articular cartilage specimens. All of the osteochondral lesions were chronic and of traumatic origin. In all cases, the fragments were utilized to evaluate the viability and proliferation of the cells, the histological appearance of the cartilage tissue and the expression of specific cartilage markers by real-time polymerase chain reaction (PCR). In the 16 patients scheduled for ACI, the expanded chondrocytes were used for chondrocyte implantation. In the other 4 patients, with lesion size $<1.5\ cm^2$, microfractures were created during the initial arthroscopic step. As a control group, 7 patients with comparable osteochondral lesions underwent the same surgery, but received chondrocytes harvested from the ipsilateral knee.

Results: According to the American Orthopaedic Foot and Ankle Scoring (AOFAS) system, patients in the experimental group had a preoperative score of 54.2 ± 16 points and a postoperative one of 89 ± 9.6 points after a minimum follow-up time of 12 months ($P < 0.0005$). The control group of patients had a preoperative score of 54.6 ± 11.7 points and a postoperative one of 90.2 ± 9.7 points at a minimum follow-up time of 12 months ($P < 0.0005$). The clinical results of the two groups did not differ significantly from each other. Chondrocytes isolated from the detached fragments were highly viable, phenotypically stable, proliferated in culture and redifferentiated when grown within the three-dimensional scaffold used for ACI. The morphological and molecular characteristics of the cartilage samples obtained from the detached osteochondral fragments were similar to those of healthy hyaline articular cartilage.

Conclusions: The good results achieved with this strategy indicate that cells derived from the lesioned area may be useful in the treatment of osteochondral defects of the talus.

Key words: Osteochondral lesion, Autologous chondrocyte implantation, Osteochondral fragment.

Introduction

Osteochondral lesions of the talus occur in approximately 6.5% of all ankle sprains, and in most cases following inversion or eversion injuries of the ankle in young and active people practicing sports[1]. The intrinsic inability of articular cartilage to heal has been widely documented[1,2] and this frequently necessitates surgical treatment[3].

The repair tissue produced after implementing surgical techniques such as chondral shaving or microfracturing bears no resemblance to the original hyaline cartilage and has poor mechanical properties[4].

Brittberg *et al.*[5] developed the autologous chondrocyte implantation (ACI) technique, which permits the repair of osteochondral lesions of the knee with a high percentage of hyaline-like cartilage tissue. Consequently, the ACI technique has been applied also to other joints, with satisfactory results particularly in the ankle[6,7]. After a follow-up time of more than 36 months in the latter, the clinical symptoms were improved, and the histological analysis of biopsy specimens revealed the formation of a repair cartilage with typical hyaline-like features[6]. Although no complications have been reported to date, one of the main problems of this surgical strategy is related to the harvesting of the cartilage slice from a healthy knee[8].

In addition, it has been shown that knee cartilage differs in composition from ankle cartilage, having a lower level of proteoglycan synthesis and less active chondrocytes[9]. These different characteristics might explain why the ankle is less affected by progressive osteoarthritis than the knee [9–11].

In order to avoid damage to a healthy knee due to the harvesting of cartilage, and to utilize cartilage with the appropriate characteristics for implantation in an ankle joint, it may be reasonable to use the detached osteochondral fragments for chondrocyte cultures.

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The aim of this study was to examine the ability of the chondrocytes harvested from detached osteochondral fragments, to proliferate and to serve as a viable source of cells for ACI in the repair of ankle cartilage defects.

Patients and methods

PATIENTS

Human articular cartilage specimens were obtained from the detached osteochondral fragments harvested from the talus of 20 patients with osteochondral lesions of this joint. The patients (11 males and 9 females) had a mean age of 30.5 ± 8 years. All of the lesions were of traumatic origin and the mean time elapsing between the traumatic event and surgery was 10 months (range: 6–20 months).

Preoperatively, all of the patients were evaluated radiographically and clinically using the American Orthopaedic Foot and Ankle Scoring (AOFAS) system. Amongst the inclusion criteria was a preoperative magnetic resonance imaging (MRI) demonstrating an osteochondral lesion and a detached osteochondral fragment. In all of the patients scheduled for surgery, the fragment was definable, still *in situ* with breached articular cartilage, and classified as stage III according to the Dipaola arthroscopic and MRI system[12]. As a control group, seven patients (mean age: 26.8 ± 9 years) with similar characteristics and who were affected by comparable osteochondral lesions underwent the same surgery, but received chondrocytes harvested from the ipsilateral knee.

The study design was approved by the Ethics Committee at our Institution and informed consent was obtained from all of the patients enrolled.

SURGICAL TECHNIQUE

In each of the 20 cases, an initial arthroscopy of the ankle was performed, which permitted fragment removal, a mild osteochondral shaving, and an accurate measurement of the lesion size. According to the guidelines recently proposed by the senior author (SG)[8], the 16 patients with a lesion size $>1.5\,cm^2$ were scheduled for ACI. In the remaining 4 cases, with a lesion size $<1.5\,cm^2$, microfracturing was performed during the initial arthroscopy.

Two parts of the detached fragments obtained from the ankles were used. One sample was utilized for histology and immunohistochemistry. The other sample was processed for chondrocyte isolation, and the cells thereby obtained were used for real-time polymerase chain reaction (PCR) and the PicoGreen® proliferation tests. As controls, two biopsy samples of healthy cartilage were obtained from the talus of two different male multiorgan donors, aged 20 and 37 years, who did not have a known history of arthritis or related pathologies.

In the 16 patients scheduled for ACI, chondrocytes were expanded from one of the fragments. These were implanted 30 days after harvesting using Hyalograft-C (Fab Advanced Biopolymers, Abano Terme, Padova, Italy).

In the control group of 7 patients, cartilage was harvested arthroscopically from the ipsilateral knee for cell culturing. As in the experimental group, chondrocytes were expanded and then implanted 30 days after harvesting using Hyalograft-C.

In both groups, the second arthroscopic step was performed using traditional anteromedial and anterolateral arthroscopic accesses. The lesion was accurately shaved and sized. Since none of the lesions had a depth >5 mm, no cancellous bone grafting was undertaken in this series[13]. The chondrocytes were directly supported on an autoadhesive hyaluronan patch, which was arthroscopically positioned over the lesion. Fibrin glue was used to improve the stability of the transplanted matrix.

CHONDROCYTE ISOLATION AND GROWTH WITHIN A THREE-DIMENSIONAL MATRIX

Chondrocytes were isolated enzymatically as previously reported[14]. Fragments of the cartilaginous tissue were cultured in Dulbecco's modified Eagle's medium [(DMEM) Life Technologies, Paisley Scotland] containing 25 mM HEPES (Sigma), penicillin [10,000 units/ml (Gibco)], streptomycin [10,000 μg/ml (Gibco)], and gentamycin [50 mg/ml (Biological Industries, Kibbutz Beth Haemek, Israel)]. Chondrocytes were isolated by sequential enzymatic digestions at 37°C: 30 min with 0.1% hyaluronidase (Sigma, St Louis, MO, USA), 1 h with 0.5% pronase (Sigma) and 1 h with 0.2% collagenase (Sigma). The isolated chondrocytes were filtered successively through sterile nylon meshes with pore diameters of 100 μm and 70 μm to remove cell and matrix debris. The filtrate was then centrifuged at 1800 rpm for 10 min. The pellet was washed twice with DMEM supplemented with 10% fetal calf serum (Biological Industries, Kibbutz Beth Haemek, Israel). The cell number and viability were assessed by ital staining with eosin. The cells were cultured under conventional monolayer conditions at 37°C in a humidified atmosphere containing 5% CO_2 for about 3 weeks (3–4 passages). The medium was changed twice a week.

To evaluate the chondrocyte redifferentiation process, cells were seeded within the same hylauronan-based three-dimensional biomaterial (Hyaff®-11, Fidia) that was used for ACI. To this end, 1×10^6 confluent cells were seeded within a 1×1 cm, 2-mm thick scaffold in a Petri dish (Becton Dickinson, Plymouth, UK) containing 150 μl of culture medium. The cells were allowed to adhere for 8 h at 37°C and before adding 2 ml of medium. The medium was changed twice a week. The constructs were analyzed after 1, 7, 14 and 21 days. Prior to seeding within the biomaterial, 1×10^6 cells were pelleted and utilized as a dedifferentiated control (dedifs).

PROLIFERATION TEST

Cell proliferation rates were determined in triplicate at different experimental times (1, 7, 14, 21 and 28 days) using the PicoGreen® dsDNA Quantitation kit (Molecular Probes, Eugene, OR, USA) for the double-stranded DNA assay. After collecting the supernatants, the adherent cells were lysed with 100 μl of lysis buffer. PicoGreen® working solution (100 μl) was then added to each well. After incubating for 5 min at ambient temperature, the fluorescence of each sample was determined using a Spectramax Gemini dual-scanning microplate spectrofluorimeter (Molecular Device, Sunnyvale, CA, USA) in the well-scan mode, at an excitation wavelength of 480 nm, an emission wavelength of 540 nm and a cut-off wavelength of 515 nm. Fluorescence readings were converted to specific values using a DNA standard curve.

QUANTIFICATION OF THE EXPRESSION CARTILAGE-SPECIFIC MOLECULES BY REAL-TIME PCR

5×10^5 cells that had been cultured in monolayers immediately after isolation were pelleted and then lysed in