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Are ankle chondrocytes from damaged fragments a suitable cell source for cartilage repair?

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SUMMARY

Objective: To characterize the post-expansion cartilage-forming capacity of chondrocytes harvested from detached fragments of osteochondral lesions (OCLs) of ankle joints (Damaged Ankle Cartilage Fragments, DACF), with normal ankle cartilage (NAC) as control.
Design: DACF were obtained from six patients (mean age: 35years) with symptomatic OCLs of the talus, while NAC were from 10 autopsies (mean age: 55 years). Isolated chondrocytes were expanded for two passages and then cultured in pellets for 14 days or onto HYAFF®-11 meshes (FAB, Italy) for up to 28 days. Resulting tissues were assessed histologically, biochemically [glycosaminoglycan (GAG), DNA and type II collagen (CII)] and biomechanically.
Results: As compared to NAC, DACF contained significantly lower amounts of DNA (3.0-fold), GAG (5.3-fold) and CII (1.5-fold) and higher amounts of type I collagen (6.2-fold). Following 14 days of culture in pellets, DACF-chondrocytes generated tissues less intensely stained for Safranin-O and CII, with significantly lower GAG contents (2.8-fold). After 28 days of culture onto HYAFF®-11, tissues generated by DACF-chondrocytes were less intensely stained for Safranin-O and CII, contained significantly lower amounts of GAG (1.9-fold) and CII (1.4-fold) and had lower equilibrium (1.7-fold) and dynamic pulsatile modulus (3.3-fold) than NAC-chondrocytes.
Conclusion: We demonstrated that DACF-chondrocytes have inferior cartilage-forming capacity as compared to NAC-chondrocytes, possibly resulting from environmental changes associated with trauma/disease. The study opens some reservations on the use of DACF-derived cells for the repair of ankle cartilage defects, especially in the context of tissue engineering-based approaches.

Introduction

For chondral and osteochondral lesions (OCLs) of the talus (ankle joint), different cell-based therapies such as the autologous chondrocyte transplantation (ACT) and matrix associated ACT are established operative procedures[1–3]. For these procedures, chondrocytes are commonly harvested from the healthy and not affected knee joint[3–5]. However, a cartilage biopsy in a joint, even if harvested from a non-load bearing site, represents an additional injury to the cartilage surface, and has been reported to be detrimental to the surrounding healthy articular cartilage[6].

Alternatively, cartilage biopsies could be harvested from healthy areas of the osteochondral defected ankle joint[7]. Indeed, promising clinical results were reported when autologous chondrocytes derived from cartilage cylinders taken from the anterior part of talus were used to restore of full-thickness cartilage defects of such joint[4]. Moreover, our recent finding that chondrocytes isolated from ankle cartilage exhibit similar proliferation ability and post-expansion tissue-forming capacity than chondrocytes isolated from knee cartilage[8] suggests that talar chondrocytes may represent a suitable cell source for the repair of cartilage lesions of the talus. The harvest of talar chondrocytes from healthy areas of defected

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ankle joints, however, could be detrimental to the function of this already injured joint[9].

To overcome the aforementioned possible problems caused by the harvesting of cartilage biopsies from healthy knee or healthy areas of the already affected ankle joint, a rational and convenient approach would be represented by the utilization of chondrocytes isolated from already detached OCL fragments which are debrided during surgery. In this regard, it was reported that viable cells can be isolated from detached osteochondral fragments from human articular joints[10,11]. Moreover, the implantation of such chondrocytes in patients was reported to yield good clinical results 12 months post-operatively[11]. However, the chondrogenic capacity of cells isolated from detached OCL fragments remains controversial. While Giannini *et al.*[11] reported that these cells have the potential to re-express or synthesize to a certain extent cartilage specific genes and proteins during culture in three-dimensional (3D) porous scaffolds, Kuroki *et al.*[12], showed that constructs generated by chondrocytes from naturally occurring OCL of the humeral head of *dogs* and cultured in agarose gel formed tissues containing inferior amount of glycosaminoglycan (GAG), type II collagen and hydroxyproline and superior amounts of type I collagen as compared to those generated by chondrocytes from healthy cartilage. Similarly, Garvican *et al.*[13] found that chondrogenic differentiation of *equine* chondrocytes from Damaged Ankle Cartilage Fragments (DACF) was inferior to that of aged-matched normal chondrocytes.

The goal of this study was thus to assess the quality of cartilaginous tissues generated by human chondrocytes harvested from the detached fragments of OCL of ankle joints (DACF), using chondrocytes derived from normal ankle cartilage (NAC) as control cells. Cells were cultured in micromass pellets or in scaffolds in clinical use for the delivery of human chondrocytes (HYAFF®-11), and the resulting tissues assessed phenotypically, biochemically and biomechanically.

Materials and methods

Cartilage biopsies

DACF were harvested from the talus of six patients (female: male = 3:3, mean age: 36, age range: 30–43 years) scheduled for surgery of symptomatic talar OCLs of this joint (Table I), following informed consent. Lesions, localized in the middle third of the medial talus edge or at the lateral side, were from traumatic origin and not associated with diagnosed instability of the ankle joint. The mean time elapsed between the traumatic event and surgery was 6.5 months, which is typically sufficient to allow for the onset of early degenerative changes in the joints (e.g., cartilage fibrillation in the border of the lesion). The OCLs, graded according to the widely used Berndt & Harty classification (grades I–V; grade I: subchondral compression; grade II: partially avulsed fragment; grade III: detached but not displaced fragment; grade IV: displaced fragments; grade V: subchondral cyst)[14] were of grade III (5/6) or grade II (1/6) (Table I). During surgery the OCL fragments were found as talar *in situ* dissecates, carefully harvested, and immediately sent from the operation room to the laboratory for further analyses.

Table I
Patient information for OCLs

	Donor 1	Donor 2	Donor 3	Donor 4	Donor 5	Donor 6
Gender	Female	Male	Male	Female	Male	Female
Age	30 Years	43 Years	39 Years	36 Years	33 Years	37 Years
Joint side	Medial	Medial	Lateral	Medial	Lateral	Medial
OCL grade (Berndt & Harty)	III	III	III	II	III	III
Interval trauma/operation	14 Months	3 Months	6 Months	6 Months	4 Months	6 Months

As control, NAC biopsies were harvested from the healthy areas of the talus of 10 cadavers (female:male = 4:6, mean age 55, range 32–79 years) in accordance with the Local Ethical Committee[8]. The chondral layers of DACF and NAC biopsies were cleaned meticulously from fibrous tissue and bone, and rinsed several times in a sterile saline solution.

DACF and NAC biopsies were chopped in small pieces which were processed in parallel for histology and immunohistochemistry (about 50 mg), for biochemistry (about 50 mg), or for chondrocyte isolation (about 100 mg) as described below.

Chondrocytes isolation and expansion

Samples derived from DACF and NAC were digested with 5 ml of type II collagenase [0.15% in basic medium – see below – supplemented with 5% fetal bovine serum (FBS)] for 22 h[8]. The isolated chondrocytes were counted using trypan blue, plated in tissue culture flasks at a density of 10,000 cells/cm^2 and cultured for two passages in Dulbecco's modified Eagle's medium (DMEM) containing 4.5 mg/ml D-glucose, 0.1 mM nonessential amino acids, 1 mM sodium pyruvate, 100 mM HEPES buffer, 100 U/ml penicillin, 100 μg/ml streptomycin, and 0.29 mg/ml L-glutamine (basic medium) supplemented with 10% FBS, 1 ng/ml of transforming growth factor (TGF)-β1 and 5 ng/ml of fibroblast growth factor-2 in a humidified 37°C/5% CO_2 incubator with medium changes twice a week, as previously described[8]. Expanded DACF- and NAC-chondrocytes were harvested for mRNA analysis or subsequently cultivated in pellets or in 3D scaffolds, according to previously established methods and as briefly described below.

Chondrocyte cultivation in pellets

The chondrogenic capacity of expanded DACF- and NAC-chondrocytes was investigated using a simple and broadly used model, namely pellet cultures in a defined serum-free medium[14]. Briefly, cells were suspended in basic medium supplemented with ITS^{+1} (Sigma Chemical, St. Louis, MO; i.e., 10 μg/ml insulin, 5.5 mg/ml transferrin, 5 ng/ml selenium, 0.5 mg/ml bovine serum albumin, 4.7 mg/ml linoleic acid), 0.1 mM ascorbic acid 2-phosphate, 1.25 mg/ml human serum albumin, 10^{-7} M dexamethasone and 10 ng/ml TGF-β1 (chondrogenic medium, CHM). Aliquots of 5×10^5 cells/0.5 ml were centrifuged at 250*g* for 5 min in 1.5 ml polypropylene conical tubes (Sarstedt, Nümbrecht, Germany) to form spherical pellets, which were placed onto a 3D orbital shaker (Bioblock Scientific, Frenkendorf, Switzerland) at 30 rpm. Pellets were cultured for 2 weeks, with medium changes twice per week, and subsequently processed for histological and immunohistochemical or biochemical analysis as described below. Each analysis was performed independently in at least two entire pellets for each primary culture.

Chondrocyte cultivation in 3D scaffolds

The ability of expanded DACF- and NAC-chondrocytes to generate neo-cartilage was also investigated by cultures in esterified hyaluronic acid non-woven meshes (HYAFF®-11, Fidia Advanced Biopolymers, Abano Terme, IT), currently in clinical use for cartilage repair[15] since such model allows more extensive cartilaginous tissue maturation than in pellets over prolonged culture times[16]. Chondrocytes were loaded statically on the scaffolds (6 mm diameter, 2 mm thick disks) at a density of