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INTRA-INDIVIDUAL COMPARISON OF HUMAN ANKLE AND KNEE CHONDROCYTES IN VITRO: RELEVANCE FOR TALAR CARTILAGE REPAIR

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ABSTRACT

Objective. As compared to knee chondrocytes (KC), talar chondrocytes (TC) have superior synthetic activity and increased resistance to catabolic stimuli. We investigated whether these properties are maintained after TC are isolated and expanded *in vitro*.

Methods. Human TC and KC from 10 cadavers were expanded in monolayer and then cultured in pellets for 3 and 14 days or in hyaluronan meshes (Hyaff®-11) for 14 and 28 days. Resulting tissues were assessed biochemically, histologically, biomechanically and by real-time RT-PCR. The proteoglycan and collagen synthesis rates in the pellets were also measured following exposure to IL-1β.

Results. After 14 days of pellet culture, TC and KC expressed similar levels of types I and II collagen mRNA and the resulting tissues contained comparable amounts of glycosaminoglycans (GAG) and displayed similar staining intensities for type II collagen. Also proteoglycan and collagen synthesis were similar in TC and KC pellets, and dropped to a comparable extent in response to IL-1β. Following 14 days of culture in Hyaff®-11, TC and KC generated tissues with similar amounts of GAG and types I and II collagen. After 28 days, KC deposited significantly larger fractions of GAG and type II collagen than TC, although the trend was not reflected in the measured biomechanical properties.

Conclusion. After isolation from their original matrices and culture expansion, TC and KC displayed similar biosynthetic activities, even in the presence of catabolic stimuli. These *in vitro* data suggest a possible equivalence of TC and KC as autologous cell sources for the repair of talar cartilage lesions.

Running headline: human knee and talar chondrocytes comparison

INTRODUCTION

Among the many surgical options for the treatment of symptomatic chondral and osteochondral lesions of the talus in the ankle joint[1], implantation of autologous chondrocytes is gaining increasing popularity, especially for critically sized defects[1-3]. For direct or matrix-associated autologous chondrocyte implantation (ACI) in the talar cartilage, cells are typically harvested from a low weight bearing area of the ipsilateral knee joint, since it is easier accessible and can guarantee larger size biopsy as compared to the affected ankle joint[2-5]. On the other hand, following substantiated considerations related to cell density in ankle cartilage and to the still controversial issue of morbidity at the harvest site in the knee, Matricali et al. proposed that "the ankle should not be excluded a priori as a possible biopsy site" for chondrocyte isolation[6]. The authors further indicated that ankle cartilage specimens could be harvested arthroscopically at the posteromedial rim of the talar bone. The use of talar chondrocytes (TC) instead of knee chondrocytes (KC), which was introduced in a recent clinical study[4], would also be supported by the potential advantage of deriving from a joint which is less susceptible than the knee to degenerative processes and has greater capacity for repair in response to damage[7]. Indeed, there is a general consensus on the fact that – as compared to KC – TC have higher synthesis rates of cartilaginous matrix proteins[7-9], as well as the capacity to organize a denser extracellular matrix, providing an increased resistance to loading and decreased sensitivity to mechanical damage[10].

For ACI-based treatment of talar lesions, TC or KC would need to be isolated from their own microenvironment and expanded *in vitro*, which is typically associated with cell dedifferentiation and loss of the original chondrocyte phenotype[11,12]. Thus, in order to advocate potential biological advantages in the use of TC as compared to KC as a source for autologous cell-based talar cartilage repair, it is necessary to assess whether following dedifferentiation TC and KC will retain the differences in biosynthetic activity and tissue forming capacity displayed in the native tissue. So far, to the best of our knowledge, only two

studies have compared isolated TC and KC, with controversial findings. Huch et al. reported that TC, following isolation and culture in alginate beads, maintained a higher synthetic activity as compared to KC[9], whereas Aurich et al., using the same culture system, found that isolated TC and KC displayed similar rates of glycosaminoglycan synthesis[13]. Importantly, both studies have been limited to the use of freshly harvested chondrocytes, and thus do not provide an indication on the biosynthetic activity and chondrogenic capacity of the cells following culture expansion, which would be relevant in the context of ACI procedures.

In the present study, we aimed at comparing TC and KC with respect to features which could have a direct implication for cell-based cartilage repair procedures, for which an in vitro cell expansion phase is required. Thus, we investigated their proliferation rate and post-expansion biosynthetic activity, capacity to generate cartilaginous tissues and response to IL-1β, a catabolic factor normally present in injured joints. For this purpose, considering the typically large inter-individual variability in chondrocyte function[14], TC and KC were isolated from different sites of the same individuals. Cells were expanded in monolayers and transferred to different 3D systems which have previously been used as standard models to investigate chondrocyte re-differentiation and synthesis of cartilage-specific extracellular matrix proteins, namely culture in micromass pellets and into 3D porous scaffolds.

MATERIALS AND METHODS

Cartilage biopsies, chondrocyte isolation and expansion

Adult human TC and KC were collected post-mortem from full-thickness biopsies of the femoral condyle or of the talus of 10 individuals (mean age: 55 years, range 32-79 years) without macroscopic signs of arthritis, following informed consent by relatives and in accordance with the Local Ethical Committee. Cartilage tissues were weighed, minced in small pieces and digested with 0.15% type II collagenase (10 mL solution/g tissue) for 22

hours. The isolated chondrocytes were expanded for two passages with Dulbecco's 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-glutamate and supplemented with 10% of foetal bovine serum (complete medium-CM), and 1 ng/mL of Transforming Growth Factor β1 (TGFβ-1), 5 ng/mL of Fibroblast Growth Factor 2 (FGF-2), and 10 ng/mL of Platelet-Derived Growth Factor type BB (PDGF-BB) (all from R&D Systems, 6 Minneapolis, MN), as previously described[15]. Chondrocytes were subsequently cultivated in pellets or in 3D scaffolds as described below.

Pellet culture

The chondrogenic capacity of post-expanded human TC and KC was investigated using a simple and broadly used model, namely pellet cultures in a defined serum-free medium[15]. Briefly, TC and KC were suspended in DMEM supplemented with ITS+1 (Sigma Chemical, St. Louis, MO), 0.1 mM ascorbic acid 2-phosphate, 1.25 mg/mL human serum albumin, 10^{-7} M dexamethasone and 10 ng/mL TGFβ1. Aliquots of 5×10^5 cells/0.5 mL were centrifuged at 1000 rpm for 2 minutes 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 3 or 14 days, with medium changes twice per week, and subsequently processed for histological, immunohistochemical, biochemical and mRNA analysis, as well as for the proteoglycan and collagen synthesis, as described below. In some experiments, pellets generated by TC and KC from 3 donors were also exposed to 1 ng/mL human recombinant Interleukin-1 beta (IL-1β) (Roche Diagnostics GmbH, Mannheim, Germany) for the last 72 hours. The IL-1β concentration was selected based on preliminary studies from a range of 0.05 to 10 ng/mL, as the one inducing a visible and reproducible loss of accumulated glycosaminoglycans (GAG) in the pellets, up to about 50% of the controls. Each analysis was performed independently in at least two entire pellets for each primary culture and expansion condition.

Culture on porous 3D scaffolds

The ability of expanded human TC and KC to generate neo-cartilage was also investigated by cultures in esterified hyaluronic acid non-woven meshes (Hyaff®-11, Fidia Advanced Biopolymers, Abano Terme, IT), since (i) the system was previously described as a reliable model for chondrocyte re-differentiation[16], (ii) the model allows more extensive cartilaginous tissue maturation than in pellets over prolonged culture times[17] and (iii) the resulting cell-scaffold constructs are currently in clinical use for cartilage repair[18]. Chondrocytes were loaded statically on the scaffolds (6 mm diameter, 2 mm thick disks) at a density of $4x10^6$ cells/scaffold. Cell-scaffold constructs were cultured in CM supplemented with 0.1 mM ascorbic acid, 10 µg/mL Insulin and 10 ng/mL Transforming Growth Factor-β3, TGFβ3, with medium was changes twice a week, as previously described[19]. After 14 or 28 days of static culture, the resulting Engineered Cartilage generated by Talar chondrocytes (ECT) and Engineered Cartilage generated by Knee chondrocytes (ECK) were analysed histologically, immunohistochemically, biochemically and biomechanically, as described below.

Measurement of [^{35}S]SO_4 and [^{3}H]proline incorporation

The proteoglycan and collagen synthesis of the cultured pellets was measured by assessing the incorporation of [35S]SO4 and [3H]proline for a period of 24h as described previously[20]. After 3 or 14 days of culture, pellets were incubated in the presence of both [35S]SO4 (1 µCi/culture) to label proteoglycans and [3H]proline (1.5 µCi/culture) to label collagen. Since synthesized ECM components can either be incorporated into the culture or released to the media, matrix synthesis was determined by measuring both fractions. For the assessment of the released ECM fraction, radiolabeled proteoglycan and collagen were precipitated overnight at 4°C using respectively 100% ethanol and 70% ammonium sulphate. Subsequently, samples were centrifuged at 14,000 rpm for 30 min and the pellets resuspended in 4 M guanidine hydrochloride or 10% sodium dodecyl sulphate in Tris buffer (0.1 M, pH 7.0) respectively for proteoglycan and collagen. For the assessment of the incorporated ECM

fraction, tissue pellets were gently washed three times with PBS to remove the unincorporated isotopes. Pellets were then digested for 15 hours at 56°C with protease K (0.5 mL of 1 mg/mL protease K in 50 mM Tris with 1mM EDTA, 1 mM iodoacetamide and 10 µg/mL pepstatin-A, respectively). The incorporation of [^{35}S]SO_4 and [^{3}H]proline in culture pellet and in conditioned medium were measured in a Packard β-liquid scintillation counter with scintillation fluid (Ultima Gold, Perkin Elmer). The amount of synthesised molecules was calculated relative to the DNA content of the tissue. For this analysis cells from 3 donors were used, with triplicate pellets for each condition.

Biochemical Analyses

ECT and ECK were lyophilized and weighed. Pellets of TC and KC, ECT and ECK were then digested with protease K (0.5 mL and 1 mL of solution respectively for pellets and scaffold-based constructs) as indicated above. GAG amounts were measured spectrophotometrically after reaction with dimethylmethylene blue[21], with chondroitin sulfate as a standard. DNA was measured spectrofluorometrically using the CyQuant cell proliferation assay Kit (Molecular Probes, Eugene, OR), with calf thymus DNA as a standard[22]. GAG contents were reported as % GAG/ dry weight tissue (for ECT and ECK) or GAG/DNA (for pellets).

For the determination of collagens type I and II, constructs were lyophilized. The dried samples were fully solubilised by digestion with 2 mg/mL Tosylamide-2-phenylethyl chloromethyl ketone-treated bovine pancreatic trypsin in 50 mM Tris-HCl, pH 7.6, containing 1mM iodoacetamide, 1mM EDTA and 10 µg/mL pepstatin A, using an initial incubation of 15 h at 37ºC followed by a further 2 h incubation at 65ºC after the addition of fresh trypsin. Samples were boiled for 15 min to inactivate the enzyme[23]. Amounts of type II collagen (CII) were assayed by inhibition ELISA using a mouse IgG monoclonal antibody to denatured CII[24]. Amounts of type I collagen (CI) were assayed by inhibition ELISA using a rabbit anti-peptide antibody to CI[23].

Real-time quantitative RT-PCR assays

RNA of pellets for TC and KC was extracted using Trizol (Life Technologies, Basel, Switzerland), according to the Manufacturer's protocol. Pellets cultured in chondrogenic medium were first sonicated for 1 minute while in Trizol. RNA was treated with DNAseI using the DNA-freeTM Kit (Ambion, USA) and quantified spectrofluorimetrically. cDNA was generated from 3 μg of RNA by using 500 μg/mL random hexamers (Catalys AG, CH) and 1 μL of 50 U/mL StratascriptTM reverse transcriptase (Stratagene, NL), in the presence of dNTPs. PCR reactions were performed and monitored using the ABI Prism 7700 Sequence Detection System (Perkin-Elmer/Applied Biosystems, Rotkreuz, Switzerland). Cycle temperatures and times as well as primers and probes used for the reference gene (18-S rRNA) and the genes of interest (collagen type I and II), were as previously described[15]. For each cDNA sample, the threshold cycle (Ct) value of 18-S was subtracted from the Ct value of the target gene , to derive ΔCt. The level of expression of type I and type II collagen was calculated as $2^{\Delta Ct}$. Each sample was assessed at least in duplicate for each gene of interest.

Histological and immunohistochemical analyses

Generated pellets and constructs were rinsed with PBS, fixed in 4% formalin, embedded in paraffin, and cross-sectioned (5 μm thick for pellets and 7 μm thick for constructs). Sections were stained with Safranin-O for sulfated glycosaminoglycans (GAG).

Sections were processed for immuno-histochemistry using an antibody against type II collagen (II-II6B3, Hybridoma Bank, University of Iowa, USA), as previously described[25].

Biomechanical analysis

Mechanical tests on constructs were conducted in a standard miniature test instrument in unconfined compression (Synergie 100, MTS Systems Corp., Eden Prairie MN, USA) to measure the Equilibrium modulus (E_{EQ}) and the Pulsatile dynamic modulus (E_{PD}), as previously described in detail[19]. Briefly, E_{EQ} was determined from a linear regression of the data pairs of equilibrium stress / incremental strain, following application of five incremental strains of 5% and computation of the corresponding equilibrium stress. E_{PD} was calculated as

the slope of the stress/strain curve, after exposing specimens to five cycles of compressive loading/unloading at 0.17 mm/s, reaching a strain of 20% and with each strain period followed by a no-load period of time equal to that for loading / unloading.

Statistical analysis

Unless otherwise stated, values are presented as mean ± standard error of measurements from 10 independent experiments, using cells from 10 donors. Statistical analyses were performed using the Sigma Stat software (SPSS Inc., Version13). Differences among experimental groups in the comparison between TC and KC were assessed by two tailed Wilcoxon test, and considered statistically significant with $p < 0.05$.

RESULTS

Proliferation rate of human ankle and knee chondrocytes.

Although native ankle cartilage has been reported to have a higher cellularity than knee cartilage[7,10,26], we found no significant difference between the cell yields of native cartilage harvested from the talus or the femoral condyle of the same individuals ($3.3\pm0.68 \times 10^6$ and $3.6 \pm 0.45 \times 10^6$ cells/g tissue respectively). It is possible that other factors beyond the tissue cellularity (e.g., efficiency of cell extraction from the extracellular matrix, or survival capacity of the cells during enzymatic treatment) might have accounted for our finding.

In the time required to reach the second confluence (i.e., 13-19 days), TC and KC displayed similar proliferation rates (0.55 ± 0.05 and 0.58 ± 0.03 doublings/day respectively) and underwent a similar number of doublings (7.7 ± 0.5 and 8.0 ± 0.2 respectively). At the end of the expansion phase, both TC and KC exhibited an elongated fibroblastic morphology, characteristic of de-differentiated chondrocytes (data not shown).

Post-expansion chondrogenic capacity in pellets

Accumulation of cartilage specific proteins

After 14 days of culture in pellets, expanded TC and KC generated hyaline-like cartilaginous tissues, with similar staining intensity for GAG and collagen type II in pellet pairs generated by the cells from the same donors (Fig. 1). Biochemical analysis quantitatively confirmed that pellets generated by TC contained GAG/DNA contents similar to those generated by KC (13.9 ± 3.2 and 12.1 ± 1.6 µg/µg respectively) (Fig. 2A). RT-PCR analysis further characterized that collagen type I and II mRNA expression was comparable in pellets generated by TC and KC (Fig. 2B). The relatively high expression of type I collagen is rather typical for chondrocytes cultured in this model for two weeks and should not be attributed to an unsuccessful cell re-differentiation, since the ratio of type I / type II collagen mRNA expression of post-expanded chondrocytes has previously been shown to strongly decrease following pellet culture[27,28].

Synthesis of cartilage specific proteins

Proteoglycan and collagen synthesis increased between 3 and 14 days of pellet culture to a similar extent for TC (6.4-fold and 1.5-fold respectively) and KC (7.8-fold and 1.2-fold respectively), remaining at comparable levels (Fig 2C-D). The released fractions of proteoglycan and collagen were rather limited, averaging less than 5% of the total amount of the newly synthesized molecules.

Response to IL-1β

Pellets at different stages of maturation (i.e., after 3 and 14 days of culture) were exposed to IL-1β for 72 hours. In 3-day pellets, for both TC and KC, IL-1β treatment resulted in extensive tissue degeneration, as evidenced histologically by the reduction of extracellular matrix and the appearance of necrotic cells (Fig 3A), as well as biochemically by the reduced amounts of GAG and synthesis of proteoglycan and collagen, down to the minimal levels of detection (Fig 3B-D). In 14-day pellets generated by both TC and KC, the exposure to IL-1β resulted in loss of cartilaginous matrix, as evidenced histologically by a reduced Safranin O staining intensity, as well as biochemically by a statistically significant reduction of GAG

contents (1.9- and 1.6-fold respectively), synthesis of proteoglycans (7.0- and 16.0-fold respectively) and synthesis of collagens (4.2- and 3.7-fold respectively) (Fig 4). Importantly, TC and KC pellets reacted in an almost identical fashion and extent to IL-1β, at both stages of maturation.

Post-expansion chondrogenic capacity in 3D scaffolds

After 14 days of culture in Hyaff-11® meshes, the resulting engineered cartilage based on talar chondrocytes (ECT) and engineered cartilage based on knee chondrocytes (ECK) were only faintly stained for GAG and collagen type II (data not shown). Instead, after 28 days of culture, ECT and ECK displayed regions intensely stained for GAG and collagen type II. At this stage, ECT appeared to be stained at lower intensity and uniformity for both extracellular matrix proteins as compared to ECK (Fig. 4).

Biochemical analysis generally confirmed the histological observations. Following 14 days of culture, similar GAG, type I and type II collagen contents were measured in ECT and ECK (Fig. 5A). The increase in the GAG content as a % of dry weight from 14 to 28 days of culture was larger for ECK than ECT (2.6- and 1.8-fold respectively), resulting in significantly higher final GAG contents (1.6-fold) in tissues formed by KC (Fig. 5A). Interestingly, type II collagen content did not increase with culture time in ECT, whereas it increased by 2.2-fold in ECK, resulting in double amounts in ECK than in ECT (Fig. 5A). Between 14 to 28 days of culture, type I collagen content increased to a similar extent in ECK and ECT, reaching similar levels at the latest time point (Fig. 5A). At 28 days, the type II / type I collagen ratio was 0.39 ± 0.16 and 0.92 ± 0.26 respectively in ECT and ECK, indicating a more fibrocartilaginous nature of the tissues generated by TC.

Interestingly, the measured biomechanical properties of tissues did not capture the biochemical differences (Fig. 5B). In fact, equilibrium modulus (E_{EQ}) and dynamic pulsatile modulus (E_{dyn}) were comparable in ECT and ECK at 2 weeks culture, significantly increased between 14 and 28 days of culture to a similar extent in ECT and ECK (E_{EQ}: 1.8- and 1.9-

fold, E_{dyn}: 1.9- and 1.4- fold respectively) and thus reached similar levels at the end of the culture.

DISCUSSION

In this study we compared proliferation capacity, post-expansion biosynthetic activity, chondrogenic ability and response to the catabolic factor IL-1β of human talar chondrocytes (TC) and knee chondrocytes (KC) from the same individuals. Cells isolated from the different cartilage tissues proliferated at a similar rate in monolayer culture and, when induced to re-differentiate in 3D pellets, synthesised and accumulated comparable amounts of GAG and type II collagen. Moreover, in response to IL-1β, TC and KC cultured in pellets reduced synthesis and accumulation of the main cartilage-specific macromolecules in a similar fashion and extent. Finally, following culture for 28 days in Hyaff® 11 scaffolds, TC formed cartilaginous tissues with lower GAG and collagen type II contents as compared to KC, but with similar biomechanical properties.

Although in the present study the behaviour of chondrocytes from native talar and knee cartilage tissues was not investigated, other groups previously reported that primary, non-expanded TC have a higher proteoglycan[7,8] and collagen synthesis[9] than KC, as well as a superior resistance to the catabolic cytokine IL-1β[8,13]. Together with those findings, our results (i) indicate that such differences are lost when chondrocytes, isolated from their original matrices, are de-differentiated by expansion and subsequently induced to re-differentiate in pellet cultures, and (ii) suggest a potential critical role of the native tissue environment (e.g., composition and organization of the extracellular or pericellular matrix) in determining the properties of KC or TC.

The capacity of de-differentiated TC and KC to generate 3D cartilaginous tissues was also tested in non-woven meshes made of esterified hyaluronic acid (Hyaff®-11), a model allowing more extensive tissue maturation than pellets[17]. Contrarily to the results obtained in pellets,

KC cultured in such scaffolds accumulated superior amounts of GAG and type II collagen than TC. Since this difference was not observed after 14 days in culture and appeared only after 28 days, the apparent discrepancy could highlight that – as compared to KC – expanded TC have an impaired capacity of extensive maturation, which cannot be discriminated in the pellet culture system. Considering the higher cell-to-cell contacts in pellets as compared to the scaffold-based cultures, the superior deposition of cartilage-specific proteins by KC as compared to TC in Hyaff®-11 meshes could alternatively be due to the fact that chondrogenesis of KC is less cell density-dependent. Yet another explanation could be related to the influence on KC by the Hyaff®-11 mesh properties, which have been previously reported to specifically modulate certain KC functions (e.g., the down-regulation of some catabolic factors involved in cartilage degeneration[29]).

It is necessary to point out that in our experiments we compared TC and KC from healthy cadaveric joints. A similar investigation using TC and KC derived from biopsies of traumatic joints of patients would be more relevant for a target clinical application. In this regard, it should be considered that (i) in focally damaged ankle cartilage, an upregulation of matrix turnover was observed also at sites remote from the lesion[30], which was not seen for the knee cartilage[31]; and that (ii) chondrocytes derived from the lesioned ankle cartilage have been proposed as a valid cell source in the treatment of cartilage defects in the talus[32].

We are also aware that the models used in the present study to characterize the post-expansion chondrogenic capacity of the TC and KC (i.e., static culture in the presence of chondrogenic factors), have not been validated to directly predict the reparative ability of the cell types when implanted in the talar lesions. In the absence of an orthotopic model where to test the performance of human chondrocytes for cartilage repair beyond the patient, further studies may investigate the matrix synthesis/production of expanded TC and KC under conditions better resembling the injured joint (e.g., with application of mechanical loading in the presence of pro-inflammatory/catabolic mediators).

In conclusion, our findings indicate that differences between human TC and KC observed when cells are in their original environments are not anymore detectable following monolayer expansion. Together with the recent evidences on the ability of expanded nasal[19] and auricular chondrocytes[33] to adapt to new environments, this study thus underlines the concept of extensive plasticity of chondrocytes following de-differentiation[15]. Moreover, from a clinical standpoint, despite the convincing body of literature on the metabolic, biochemical and biomechanical differences between talar and knee cartilage tissues, our results indicate a possible equivalence of TC and KC as autologous cell sources for the repair of cartilage lesions of the talus.

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CONFLICT OF INTEREST STATEMENT

No Author has any interest that is potentially in conflict with this work

REFERENCES

1. Giannini S, Vannini F. Operative treatment of osteochondral lesions of the talar dome: current concepts review. Foot Ankle Int 2004;25:168-75.

2. Ronga M, Grassi FA, Montoli C, Bulgheroni P, Genovese E, Cherubino P. Treatment of deep cartilage defects of the ankle with matrix-induced autologous chondrocyte implantation (MACI). Foot Ankle Surg 2005;11:29-33.

3. Whittaker JP, Smith G, Makwana N, Roberts S, Harrison PE, Laing P, *et al*. Early results of autologous chondrocyte implantation in the talus. J Bone Joint Surg Br 2005;87:179-83.

4. Baums MH, Heidrich G, Schultz W, Steckel H, Kahl E, Klinger HM. Autologous chondrocyte transplantation for treating cartilage defects of the talus. J Bone Joint Surg Am 2006;88:303-8.

5. Petersen L, Brittberg M, Lindahl A. Autologous chondrocyte transplantation of the ankle. Foot Ankle Clin 2003;8:291-303.

6. Matricali GA, Dereymaeker GP, Luyten FP. The posteromedial rim of the talar dome as the site for harvesting cartilage in the ankle: an anatomic study. Arthroscopy 2006;22:1241-5.

7. Kuettner KE, Cole AA. Cartilage degeneration in different human joints. Osteoarthritis Cartilage 2005;13:93-103.

8. Eger W, Schumacher BL, Mollenhauer J, Kuettner KE, Cole AA. Human knee and ankle cartilage explants: catabolic differences. J Orthop Res 2002;20:526-34.

9. Huch K. Knee and ankle: human joints with different susceptibility to osteoarthritis reveal different cartilage cellularity and matrix synthesis in vitro. Arch Orthop Trauma Surg 2001;121:301-6.

10. Treppo S, Koepp H, Quan EC, Cole AA, Kuettner KE, Grodzinsky AJ. Comparison of biomechanical and biochemical properties of cartilage from human knee and ankle pairs. J Orthop Res 2000;18:739-48.

11. Benz K, Breit S, Lukoschek M, Mau H, Richter W. Molecular analysis of expansion, differentiation, and growth factor treatment of human chondrocytes identifies differentiation markers and growth-related genes. Biochem Biophys Res Commun 2002;293:284-92.

12. Darling EM, Athanasiou KA. Rapid phenotypic changes in passaged articular chondrocyte subpopulations. J Orthop Res 2005;23:425-42.

13. Aurich M, Mollenhauer J, Kuettner KE, Cole AA. Differential effects on IL-1 beta on human knee and ankle chondrocytes. In: Hascall VE; Kuettner KE, Eds. The Many Faces of Osteoarthritis. Basel: Birkhauers Verlang 2002;429-32.

14. Barbero A, Grogan SP, Schafer D, Heberer M., Mainil-Varlet P, Martin I. Age related changes in human articular chondrocyte yield, proliferation and post-expansion chondrogenic capacity. Osteoarthritis Cartilage 2004;12:476-84.

15. Barbero A, Ploegert S, Heberer M, Martin I. Plasticity of clonal populations of dedifferentiated adult human articular chondrocytes. Arthritis Rheum 2003;48:1315-25.

16. Gigolo B, Lisignoli G, Piacentini A, Florini M, Gobbi P, Mazzotti G, *et al*. Evidence for redifferentiation of human chondrocytes grown on a hyluronan-based biomaterial (HYAff 11): molecular, immunohistochemical and ultrastructural analysis. Biomaterials 2002;23:187-95

17. Marsano A, Millward-Sadler SJ, Salter DM, Adesida A, Hardingham T, Tognana E, *et al*. Differential cartilaginous tissue formation by human synovial membrane, fat pad, meniscus cells and articular chondrocytes. Osteoarthritis Cartilage 2007;15:48-58

18. Marcacci M, Berruto M, Brocchetta D, Delcogliano A, Ghinelli DN, Gobbi A *et al*. Articular cartilage engineering with Hyalograft C: 3-year clinical result. Clin Orthop Relat Res 2005;435:96-105

19. Candrian C, Vonwil D, Barbero A, Bonacina E, Miot S, Farhadi J, *et al*. Engineered cartilage generated by nasal chondrocytes is responsive to physical forces resembling joint loading. Arthritis Rheum 2008;58:197-208.

20. Waldman SD, Couto DC, Grynpas MD, Pilliar RM, Kandel RA. A single application of cyclic loading can accelerate matrix deposition and enhance the properties of tissue-engineered cartilage. Osteoarthritis Cartilage 2006;14:323-30.

21. Farndale RW, Buttle DJ, Barrett AJ. Improved quantitation and discrimination of sulphated glycosaminoglycans by use of dimethylmethylene blue. Biochim Biophys Acta 1986;883:173-7.

22. Handley CJ, Buttle DJ. Assay of proteoglycan degradation. Methods Enzymol 1995;248:47-58.

23. Dickinson SC, Sims TJ, Pittarello L, Soranzo C, Pavesio A, Hollander AP. Quantitative outcome measures of cartilage repair in patients treated by tissue engineering. Tissue Eng 2005;11:277-87.

24. Hollander AP, Heathfield TF, Webber C, Iwata Y, Bourne R, Rorabeck C, *et al*. Increased damage to type II collagen in osteoarthritic articular cartilage detected by a new immunoassay. J Clin Invest 1994;93:1722-32.

25. Grogan SP, Rieser F, Winkelmann V, Berardi S, Mainil-Varlet P. A static, closed and scaffold-free bioreactor system that permits chondrogenesis in vitro. Osteoarthritis Cartilage 2003;11:403-11.

26. Cole AA, Margulis A, Kuettner KE. Distinguishing ankle and knee articular cartilage. Foot Ankle Clin 2003;8:305-16.

27. Jakob M, Demarteau O, Schafer D, Hintermann B, Dick W, Heberer M, *et al*. Cell yield, proliferation, and postexpansion differentiation capacity of human ear, nasal, and rib chondrocytes Tissue Eng 2004;10:762-70.

28. Tay AG, Farhadi J, Suetterlin R, Pierer G, Dick W, Heberer M, Martin I. Specific growth factors during the expansion and redifferentiation of adult human articular chondrocytes enhance chondrogenesis and cartilaginous tissue formation in vitro. J Cell Biochem 2001;81:368-77.

29. Facchini A, Lisignoli G, Cristino S, Roseti L, De Franceschi L, Marconi E *et al*. Human chondrocytes and mesenchymal stem cells grown onto engineered scaffold. Biorheology 2006;43:471-80.

30. Aurich M, Mwale F, Reiner A, Mollenhauer JA, Anders JO, Fuhrmann RA, *et al*. Collagen and proteoglycan turnover in focally damaged human ankle cartilage: evidence for a generalized response and active matrix remodelling across the entire joint surface. Arthritis Rheum 2006;54:244-52.

31. Squires GR, Okouneff S, Ionescu M, Poole AR. The pathobiology of focal lesion development in aging human articular cartilage and molecular matrix changes characteristic of osteoarthritis. Arthritis Rheum 2003;48:1261-70.

32. Giannini S, Buda R, Grigolo B, Vannini F, De Franceschi L, Facchini A. The detached osteochondral fragment as a source of cells for autologous chondrocyte implantation (ACI) in the ankle joint. Osteoarthritis Cartilage 2005;13:601-7.

33. Gorensek M, Jaksimovic C, Kregar-Velikonja N, Gorensek M, Knezevic M, Jeras M, *et al*. Nucleus pulposus repair with cultured autologous elastic cartilage derived chondrocytes. Cell Mol Biol Lett 2004;9:363-73.

LEGEND TO FIGURES

Figure 1. Histological properties of talar chondrocytes (TC) and knee chondrocytes (KC) pellets after 2 weeks of culture.

(**A** - **B**) Safranin O and type II collagen immunohistochemical staining of representative pellets generated by TC and KC from the same donors and cultured for two weeks in chondrogenic medium. Bar = 100 μm.

Figure 2. Biosynthetic activity of talar chondrocytes (TC) and knee chondrocytes (KC) pellets after 2 weeks of culture.

(**A**) Sulfate glycosaminoglycan content normalized to the amount of DNA. (**B**) Real time reverse transcriptase-polymerase chain reaction analysis of the expression of mRNA for type I (CI) and type II (CII) collagens. Values are mean ± SEM of measurements obtained from 10 independent experiments.

Newly synthesized amounts of collagen (**C**) and proteoglycan (**D**), measured by the incorporation of respectively [^{3}H]proline and [^{35}S]SO_4 in pellet maintained in culture for 3 and 14 days. The upper and lower parts of the columns are related respectively to the newly synthesized molecules released in the medium or accumulated in the extracellular matrix.

Values are mean ± SEM of measurements obtained from 3 independent experiments. * = significantly different from the earlier time point of the same cell source.

Figure 3. Respose to IL-1β by tissues generated by talar chondrocytes (TC) and knee chondrocytes (KC) at different stages of maturation

(**A**) Safranin O stainings of representative pellets generated by TC and KC from the same donors. Bars = 100 μm. (**B**) Sulfate glycosaminoglycan content normalized to the amount of DNA in pellets maintained in culture for a total of 14 days where the last 72 hours with (IL-1) or without (CTR) IL-1β. Newly synthesized amounts of collagen (**C**) and proteoglycan (**D**), measured by the incorporation of respectively [^{3}H]proline and [^{35}S]SO_4, in pellets maintained in culture 14 days. The upper and lower parts of the columns are related respectively to the newly synthesized molecules released in the medium or accumulated in the extracellular matrix. Values are mean ± SEM of measurements obtained from 3 independent experiments. * = significantly different from CTR.

Figure 4. Histological properties of the engineered cartilage generated by talar chondrocytes (ECT) and engineered cartilage generated by knee chondrocytes (ECK) tissue after 4 weeks of static culture

(**A - B**) Safranin O and type II collagen immunohistochemical stainings of representative ECT or ECK. Bar = 100 μm.

Figure 5. **Biosynthetic activity and biomechanical properties of engineered cartilage generated by talar chondrocytes (ECT) and engineered cartilage generated by knee chondrocytes (ECK) tissues after 2 and 4 weeks of static culture.**

(**A**) Amounts of glycosaminoglycan (GAG), type I and type II collagen accumulated, expressed as a percentage of tissue dry weight. (**B**) Equilibrium modulus and dynamic pulsatile modulus of ECT and ECK constructs. The plotted line indicates the biomechanical properties of the cell-free Hyaff-11 scaffold. Values are mean ± SEM of measurements

obtained from 10 independent experiments. * = significantly different from the earlier time point of the same cell source; ° = significantly different from ECT.