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Enhanced cell-induced articular cartilage regeneration by chondrons; the influence of joint damage and harvest site

L.A. Vonk † *, T.S. de Windt †, A.H.M. Kragten †, M. Beekhuizen †, S.C. Mastbergen ‡, W.J.A. Dhert † §, F.P.J.G. Lafeber ‡, L.B. Creemers †, D.B.F. Saris † ||

† *Department of Orthopaedics, University Medical Center Utrecht, Utrecht, The Netherlands*
‡ *Department of Rheumatology & Clinical Immunology, University Medical Center Utrecht, Utrecht, The Netherlands*
§ *Faculty of Veterinary Medicine, Utrecht University, Utrecht, The Netherlands*
|| *Tissue Regeneration, MIRA Institute, University Twente, Enschede, The Netherlands*

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SUMMARY

Objective: Interactions between chondrocytes and their native pericellular matrix provide optimal circumstances for regeneration of cartilage. However, cartilage diseases such as osteoarthritis change the pericellular matrix, causing doubt to them as a cell source for autologous cell therapy.
Methods: Chondrons and chondrocytes were isolated from stifle joints of goats in which cartilage damage was surgically induced in the right knee. After 4 weeks of regeneration culture, DNA content and proteoglycan and collagen content and release were determined.
Results: The cartilage regenerated by chondrons isolated from the damaged joint contained less proteoglycans and collagen compared to chondrons from the same harvest site in the nonoperated knee ($P < 0.01$). Besides, chondrons still reflected whether they were isolated from a damaged joint, even if they where isolated from the opposing or adjacent condyle. Although chondrocytes did not reflect this diseased status of the joint, chondrons always outperformed chondrocytes, even when isolated from the damaged joints ($P < 0.0001$). Besides increased cartilage production, the chondrons showed less collagenase activity compared to the chondrocytes.
Conclusion: Chondrons still outperform chondrocytes when they were isolated from a damaged joint and they might be a superior cell source for articular cartilage repair and cell-induced cartilage formation.

Introduction

Hyaline articular cartilage is a highly specialized tissue, which lines the bones within synovial joints such as the knee. It allows for smooth movement and is able to withstand compressive and shear forces and distribute them onto the subchondral bone[1–3]. Cartilage consists of an extracellular matrix (ECM) and a relatively small number of cells i.e., chondrocytes. This ECM is primarily composed of collagens (mainly type II collagen), proteoglycans, glycosaminoglycans, and glycoproteins[4].

When injured, articular cartilage has a limited potential of endogenous repair. Due to this characteristic, especially in weight bearing areas, damage results in an on-going process of progressive tissue damage. Untreated cartilage defects eventually lead to osteoarthritis[1–3,5].

Currently, autologous chondrocyte implantation (ACI) is increasingly being considered the standard of care for larger cartilage lesions[5,6]. In an ACI procedure, chondrocytes are traditionally harvested from a non-load bearing site in the affected knee during an arthroscopy. The chondrocytes are released from the matrix by enzymatic digestion, expanded and subsequently implanted in the focal cartilage defect. Although new tissue is formed in the defect and clinical results appear to be good, the quality of the regenerated tissue leaves room for improvement[7,8]. The repair site often contains fibrocartilaginous tissue, which is inferior to hyaline cartilage. This fibrous tissue has suboptimal mechanical properties compared to hyaline cartilage and is therefore more prone to degeneration.

Several studies have shown that chondrons produce cartilage of better hyaline quality as compared to fully isolated chondrocytes *in vitro*. Chondrons are defined as chondrocytes with their

* Address correspondence and reprint requests to: L.A. Vonk, Department of Orthopaedics, University Medical Center Utrecht, PO Box 85090, 3584 CX Utrecht, The Netherlands.
E-mail address: l.a.vonk@umcutrecht.nl (L.A. Vonk).

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pericellular matrix[9–11]. This pericellular matrix consists of relatively high levels of type VI collagen compared to the interchondron ECM, but also contains type II, III, IX and XI collagen, hyaluronan, proteoglycans, glycosaminoglycans and glycoproteins[12–18].

It has been hypothesized that the profound interactions between chondrocytes and their native pericellular matrix provides a more favorable condition for cartilage matrix production, than chondrocytes without such a cell-matrix interaction[11]. In addition, it has been shown that the pericellular matrix protects chondrocytes from collagen-induced collagenase (MMP13) expression, an enzyme that can efficiently degrade collagen from the cartilage ECM, and thus leads to matrix collagen degradation[19,20]. From that point of view, it would be preferable to use chondrocytes with their pericellular matrix (chondrons) for cartilage repair instead of fully isolated chondrocytes.

Recently, there has been considerable debate on the optimal location to harvest cells for an ACI. Where it is not ideal to harvest from intact tissue as performed now, it may be that the affected cartilage from the rim of a defect has different regenerative capacities[21]. Besides, changes in the pericellular matrix have been suggested to represent one of the earliest identifiable matrix changes associated with cartilage diseases[20,22–26]. This would contra-indicate the use of chondrons from affected cartilage for articular repair strategies. Hence, the question is whether chondrons from damaged articular cartilage are still able to outperform isolated chondrocytes from these areas and maybe even from healthy areas.

In this study, we investigated whether chondrons obtained from damaged articular cartilage still have a higher regenerative capacity when compared to chondrocytes without their pericellular matrix. Chondrons and chondrocytes were isolated from cartilage of the stifle joints of goats 20 weeks after surgical induction of cartilage damage of the medial femoral condyle of the right knee (according to the Groove model)[27–29]. The left knee was left untouched. Chondrons and chondrocytes isolated from different locations (affected and non-affected) were compared.

Materials and methods

Animals and model

Nine skeletally mature milk goats (72.9 ± 2.9 kg, age 2.3 ± 0.2 years, all female) were obtained from a commercial Dutch breeder. During the entire experiment the animals were housed in two groups of four and five animals each and were allowed to walk freely in pens of approximately 20 square meters. There were no dietary restrictions and the goats had access to water ad libitum. The Utrecht University Medical Ethical Committee for animal studies approved the experiment (DEC2009.III.01.002). After a few weeks of acclimation, cartilage damage was introduced on the medial femoral condyle of the right stifle joints according to the Groove model[27–29]. Under general anesthesia, surgery was performed through a 3–5 cm medial incision close to the ligamentum patellae. Cartilage of the weight bearing area of the medial femoral condyle was surgically damaged. A maximum of 10 diagonal and longitudinal grooves were made with a K-wire that was bended at 0.4 mm of the sharp triangular tip, preventing damage of the underlying subchondral bone. Grooves were made under visual control in utmost flexion of the knee assuring no harm was done to the opposing tibial plateau. Afterwards, the synovial tissue, joint capsule and skin were sutured separately according to their anatomical layers. Pain medication and antibiotics were supplied until 3days post-operatively. One day postoperative, all animals were fully active and showed normal behavior. The contralateral stifle joints were left untouched and served as paired inter-articular controls. Twenty weeks (5 months) later the goats were euthanized and both hind limbs amputated. By scoring stained sections[30] according to the Osteoarthritis Research Society International (OARSI) criteria[31], it was verified that the medial femoral condyle of the right knee showed clear signs of cartilage damage and the opposing tibial plateau showed less severe damage, while both the lateral compartments and non-operated knees showed no damage[32].

Isolation and culture of cells

Directly after removal of both hind limbs from the goats, the joints were opened under laminar flow conditions and full thickness cartilage samples, excluding the underlying bone, were taken from predefined locations. Articular cartilage samples were taken from the medial femoral condyles of both legs, lateral femoral condyles of both legs, medial sides of the tibial plateaus of both legs and lateral sides of the tibial plateaus. Samples from the respective joint locations were analyzed separately. Subsequently, one half from each location (randomly taken) was used for chondrocyte isolation and the other half for chondron isolation.

For chondrocyte isolation, the tissue fragments were subjected to sequential treatments of Dulbecco's modified Eagle's medium (DMEM, Gibco, Paisley, UK) supplemented with 1% fetal bovine serum (FBS, HyClone, Logan, UT), 100 U/ml penicillin, 100 μg/ml streptomycin (all from Gibco) and 2.5% (w/v) Pronase E (Sigma, St. Louis, MO) for 1 h, then with DMEM supplemented with 25% FBS, 100 U/ml penicillin, 100 μg/ml streptomycin and 0.125% (w/v) collagenase (CLS-2, Worthington, Lakewood, NJ) for 16 h at 37°C.

For chondron isolation, minced cartilage was digested with 0.3% (w/v) dispase (Gibco) plus 0.2% (w/v) collagenase in phosphate buffered saline (PBS, Gibco) supplemented with 100 U/ml penicillin and 100 μg/ml streptomycin for 5 h at 37°C[33].

The cells were filtered through a 100 μm cell strainer (BD Biosciences, San Diego, CA) and washed. The cells that were not directly processed for analysis were seeded at a density of 1.6×10^6 cells/cm^2 on Millicell filters (0.4 μm polytetrafluoroethylene (PFTE) (Millipore, Bedford MA)) that were precoated with type II collagen (type II collagen from chicken sternal cartilage (Sigma))[34,35]. The filters were cultured in DMEM supplemented with 10% FBS, 100 U/ml penicillin, 100 μg/ml streptomycin and 50 μg/ml ascorbate-2-phosphate (Sigma).

Cultures were incubated at 37°C in 5% CO_2 and culture media were renewed every 3 days.

Pericellular matrix staining

Cytospin slides were prepared using freshly harvested chondrons and chondrocytes by cytocentrifugation (500 rpm, 5 min) (Thermo Fisher Scientific Inc., Waltham, MA) and fixed for 30 min with a 4% buffered formaldehyde solution. The cytospins were stained using Safranin O-fast green[30].

Papain digestion

After 4 weeks of culture, samples were digested at 60°C for 18 h in a papain enzyme solution consisting of 5 mM L-cysteine, 50 mM Na_2EDTA, 0.1 M NaAc, pH 5.53 with 2% (v/v) papain (Sigma).

Proteoglycan analysis

To analyze the proteoglycan content of the regenerated cartilage tissue, a dimethylmethylene blue (DMMB) spectrophotometric analysis was performed[36]. The papain digest or medium sample was mixed with the DMMB solution and the absorbance was read