

Cartilage preservation and possible treatments of articular cartilage replacement in orthopedic surgery

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PhD. thesis



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1. Introduction

Full-thickness cartilage lesions in adults caused by mechanical injuries or diseases, are common problems in orthopedic surgery treating locomotor disorders. The mature cartilage is an avascular, bradytroph tissue, therefore the cartilage nutrition is only available via diffusion from the synovial fluid, and the chondrocytes show slow metabolic activity. The articular cartilage is vulnerable due to its special position, the numerous forces reaching the chondral surface and the intensive mechanical strain. In inverse proportion to its vulnerability, the regenerative healing capacity of articular cartilage is strongly restricted. Once a cartilage lesion occurs on a weight-bearing surface of a joint, it increases and the whole progress extends not only in size but also in depths reaching the subchondral bony surface resulting in osteoarthritis. The cartilage lesions predominantly affect the weight-bearing articular surfaces of joints, therefore the development of chondral or osteochondral defects is a great burden to locomotor patients.

In the group of articular cartilage lesions two different clusters are distinguishable: cartilage degeneration often occurring in elderly with osteoarthritis, and focal cartilage defects in younger population created by traumatic impact.

The currently practiced surgical treatments are used to repair cartilage defects by either delivering chondrogenic cells, or readily-formed cartilage tissue to the defect site. To the former, regenerative group belong the periosteal flapping technique, the abrasion arthroplasty, bone marrow stimulation, or the microfracture. To the latter, reconstructive group belong the osteochondral auto/allografting, and mosaicplasty.

The autologous chondrocyte implantation (ACI), which also fits into the reconstructive group, is the first real biological therapeutical option for cartilage repair, based on chondrocyte tissue engineering. The procedure consists of two surgical interventions. For the first step a cartilage biopsy is obtained from a non-weight-bearing surface of the articular cartilage. The cartilage matrix of the biopsy specimen is gradually removed enzymatically, the chondrocytes are isolated with collagenases, and then expanded under certain circumstances using special culture media in tissue engineering laboratory. Parallel with the cell expansion the chondrocytes might lose their cartilage-specific phenotype, their special morphological and functional features,

and they become more and more fibrocyte-like, and later they dedifferentiate into fibroblasts. Knowing this fact, the continuous microscopical and transcriptional quality control of the tissue engineering is inevitable. Microscopical analysis is used for the assessment of morphological changes, and gene expression profiling for the transcriptional bias. The most important chondrocyte-specific gene expression markers are the collagen type II. (COL2A1), aggrecan, and cartilage oligomeric matrix protein (COMP).

The viable, quality controlled chondrocyte cell suspension reaching the necessary cell number, is able to be reimplanted onto the cartilage defect with different securements, and different techniques during a second surgical intervention.

During the I. generation ACI method, the expanded chondrocytes are injected beneath a periosteal flap or collagen membrane, whereas the cartilage cells are seeded onto the surface of a biodegradable matrix according to the II. generation ACI technique. These three-dimensional matrices act as a carrier and active environment for the chondrocytes. ACI method can only be used to repair the chondral defects, when its application suits all of the strict requirements and criteria. Similar to that, rigorous follow-up is also strongly advisable, with clinical functional tests, MRI examination, second-look arthroscopy, or histological assessment of cartilage biopsy obtained from the repair site.

Under normal circumstances, there is a delicate balance between the cartilage matrix synthesis and degradation, so that the cartilage tissue is able to maintain its original physiological function. The apoptosis, as programmed cell death, plays an important regulating role to maintain the specific entity of articular cartilage. It is particularly essential to avoid or minimize the cell death in chondrocyte cell cultures, and also in replanted cartilage units to maintain the efficacy of cartilage repair methods.

2. Aims of the present study

- establishment of biotechnological laboratory, cell culture experiments on porcine and human chondrocytes, chondrocyte tissue engineering
- quality control of chondrocyte cell cultivation, microscopical analysis of expanded chondrocytes, monitoring the chondrocyte gene expression profile with PCR method
- comparative investigation of the legal background and implantation criteria of ACI method in legal systems of different countries
- establishment of a hungarian professional guideline for ACI method
- chondrocyte viability experiments with local anesthetics, glucocorticoids and their combination under in vitro and ex vivo circumstances, in chondrocyte cell cultures and osteochondral samples
- experiments in three-dimensional matrices seeded with chondrocytes, assessment of characteristics, and spatial arrangement of porcine and human chondrocytes
- I. and II. generation ACI experiments in ovine and porcine animal models
- implantation of multiply incised, surface-augmented chondral allograft in porcine models, construction of a new designed cartilage harvester and cartilage incisor
- comparative animal experiments with different cartilage repair methods
- introduction of ACI method in humans

3. Materials and Methods

3.1. Chondrocyte tissue engineering, quality control of chondrocyte cell cultivation

Our animal experiences were carried out in the Department of Animal Nutrition at Kaposvár University. We obtained the articular cartilage specimens from the non-weight-bearing surface of intact femur condyles of mature ovine and porcine models. The human chondrocytes originated from cadaveric specimens from tissue bank, and also from specimens obtained intraoperatively in Department of Orthopedics at University of Pécs. We set our cell expansion laboratory in Department of Immunology and Biotechnology, where we started a series of cell expansions from cartilage specimens obtained from animal models and humans.

To assess morphological changes of the expanded chondrocytes histologic evaluation was carried out. To monitor gene expression characteristics of chondrocytes we obtained total RNA samples from the cells, and levels of chondrocyte-specific COL2A1, aggrecan, and COMP gene activity were measured by reverse transcriptase PCR method.

3.2. Chondrocyte viability tests with steroids and local anesthetics

To determine and evaluate chondrocyte apoptosis and necrosis under in vitro and ex vivo circumstances, we used chondrocyte cultures and osteochondral specimens. Randomly allocated groups of these two kinds of specimens were treated with 10 different agents, glucocorticoids and local anesthetics alone or in various combinations, as follows: betamethasone, prednisolone, lidocaine, bupivacaine, and ropivacaine alone, and betamethasone/lidocaine, betamethasone/bupivacaine, betamethasone/ropivacaine, prednisolone/lidocaine in combinations. Phosphat-buffered saline served as control.

The cell death in chondrocyte cultures was evaluated by flow cytometry after staining with fluorescein isothiocyanate (FITC)-labeled annexin-V and propidium-iodide (PI). Apoptosis and necrosis appearing in osteochondral specimens were investigated by TdT-mediated dUTP nick-end labeling (TUNEL) assay.

3.3. Chondrocyte features on three-dimensional matrices, I. and II. generation ACI animal experiences

Porcine and human chondrocyte cell suspensions after the fourth cell passage reaching the appropriate number of $1-2 \times 10^7$, were seeded onto the surface of three-dimensional Soft PGA matrix, then fixed with fibrinogene/thrombin. The viability and spatial arrangement of chondrocytes were assessed with microscopic evaluation, whereas the gene expression activity of chondrocyte-specific markers, such as COL2A1, aggrecan, and COMP, together with dedifferentiation marker collagen type I. were measured with PCR method.

In the first part of our animal experiences we examined mature, female ovine models, later we continued our investigations with the replacement of chondral defects on the weight-bearing articular surfaces of medial femur condyles of porcine models. During the I. generation ACI method the chondrocyte cell suspension was injected beneath a periosteal flap, which was perviously harvested from the tibial surface of porcine models, and tightly secured onto the cartilage defects with stiches along with Tissucol fibrin glue. Oppositely to that, the chondrocyte cell suspension according to the II. generation ACI, was seeded onto the surface of three-dimensional Soft PGA matrix, and the whole matrix was replanted onto the defect site. The repair tissue was evaluated after 24 weeks with both macroscopic and microscopic analysis. For macroscopic assessment purposes we used the International Cartilage Repair Society (ICRS) Evaluation Scale, while the microscopic evaluation consisted of analysis of osteochondral specimens obtained from the defect site according to the ICRS- I. Visual Assessment Scale, after haematoxyline-eosine (HE), and periodic acid-Schiff (PAS) staining.

3.4. Multiply incised, surface-enhanced cartilage allograft studies using specially designed cartilage harvester and incisor

We obtained our cartilage specimens with a newly designed and patented instrument, which was capable to follow the natural cartilage surface more precisely, due to a curvilinear cutting blade. The deep zone of full thickness articular cartilage grafts was multiply parallel incised in every 200 μm , with a specially designed cartilage incisor. The incisions did not go through the entire cartilage, not compromising the integrity of the superficial cartilage layer. With this cartilage harvesting method we got a

deformable base, whose surface was significantly augmented. The multiply-incised chondrografts were secured onto the surface of primary chondral defects, and the repair tissue were investigated macroscopically and histologically after 6, and 24 weeks with ICRS Macroscopic Evaluation Scale and ICRS- I. Visual Assessment Scale, after HE and PAS staining.

3.5. Comparative animal experiments with different cartilage repair methods

In our animal studies we investigated the results of the following cartilage repair methods: microfracture, I. and II. generation ACI techniques, and multiple-incised, processed chondrograft. In the control group, the created full-thickness cartilage defect was left untreated. 24 weeks after the surgical interventions the repair tissue was assessed macroscopically according to the ICRS recommendations. The microscopic assessment of osteochondral biopsy samples was based on the ICRS Visual Histological Assessment Scale.

3.6. Introduction of autologous chondrocyte implantation method in human

For the first human ACI intervention, we replaced a large chondral defect on the medial femur condyle of the knee joint of a 33-year-old, male sportsman with II. generation ACI method. We previously harvested cartilage fragments from the non-weight-bearing area of the articular surface, then after a cell expansion, the chondrocyte cell suspension was seeded onto a carrier PGA matrix, which was reimplanted and secured onto the cartilage defect with stiches and fibrin glue. After 6, 12, and 24 months we evaluated the affected knee joint with clinical functional tests according to the Lysholm score, furthermore we assessed the integration of the repair tissue with MRI method at 6, and 12 months postoperatively.

4. Results

4.1. Chondrocyte tissue engineering, quality control of chondrocyte cell cultivation

At the beginning of our animal experiments we started to expand ovine, later porcine and human chondrocyte cell cultures. The chondrocyte suspensions originated from ovine and porcine models, reached the appropriate cell density in a shorter time,

whereas human chondrocytes showed more reliable and even passage characteristics. Our morphological analysis showed a gradual transformation of chondrocytes into fibroblasts. The relative gene expression of the main chondrocyte-specific marker COL2A1 revealed a gradual and significantly decreasing tendency, particularly after the fourth cell passage. Similar to this finding, the relative gene expression level of COMP and aggrecan also decreased, whereas the expression of beta-actin was present in a relative constant level.

4.2. Chondrocyte viability tests with steroids and local anesthetics

4.2.1. In vitro results on chondrocyte cell cultures

Chondrocytes exposed to lidocaine showed apoptosis reaching almost 20%, since the ropivacaine treatment alone showed approximately 40% of cell necrosis by 24 hours. Bupivacaine was the most cytotoxic among the three anesthetics, because it induced almost 100% cell necrosis after 24 hours of exposure. The two distinct steroid preparations, betamethasone and prednisolone showed similar characteristics in inducing cell death, reaching 20% of cell death by 24 hours. The combination of betamethasone and lidocaine increased chondrocyte apoptosis even after 6 hours when compared with betamethasone or lidocaine administration alone. This combination also increased the number of necrotic cells after 24 hours of exposure, because 83% of the cells were necrotic by that time. The combination of prednisolone with lidocaine did not cause a major increase in apoptosis or necrosis rate after 2- or 6-hour exposure. However the percentage of apoptotic cells detected in cell cultures at 24 hours of exposure reached 65%, with an additional 25% of necrotic cells. At each detection point, in cases of combined use of betamethasone-bupivacaine and betamethasone-ropivacaine the necrotic rate was the highest, almost 100%.

4.2.2. Ex vivo results on osteochondral specimens

In the osteochondral specimens, administration of glucocorticoids and local anesthetics alone affected only the superficial layer of the specimens. TUNEL assay of specimens treated with lidocaine for 24 hours showed the presence of several dead cells (16% of all cells) in the superficial zone, whereas chondrocytes situated in the

deeper zones showed consistently high viability. Betamethasone treatment led to minor cytotoxic activity (3%), whereas after prednisolone exposure TUNEL-positive cells (22% of all cells) were situated predominantly in the superficial 1/3 of the cartilage, but not in the deeper zones. Prednisolone-lidocaine combined exposure resulted in several, randomly scattered dead cells (36% of all cells) across the whole depth of cartilage sample. The ratio of nonviable chondrocytes after betamethasone-lidocaine combination was similar to that after prednisolone-lidocaine combined treatment, however cells were affected mostly in the superficial zone. The glucocorticoid-local anesthetic combinations showed a higher cell death ratio, than administered alone in the osteochondral specimens.

4.3. Chondrocyte features on matrices, I. and II. generation ACI animal experiences

The expanded porcine and human chondrocyte cell suspensions were seeded onto carrier matrices. The chondrocytes revealed to be associated with piles of the matrix when assessed microscopically. The spatial distribution of the chondrocytes showed a three dimensional arrangement. The relative gene expression levels of COL2A1, COMP, and aggrecan chondrocyte markers decreased with the increasing number of cell passages before the cell-seeding. In contrast to that, after seeding we found increasing levels of previous markers with parallel decrease in collagen type I.

We started our ACI investigations primarily on ovine models, but we found that the unsatisfactory cartilage thickness and the tight articular situation of ovine knee joints made it impossible to apply the ACI technique.

We carried out macroscopic evaluation 24 weeks after chondrocyte implantations in porcine models. Our observations in our I. generation ACI groups showed, that the defects were filled in approximately 80-85%, the repair tissue was integrated with less than 1 mm demarcation border, although the defect site comprised several fissures. Our II. generation ACI results revealed that the defect completion was 90-95%, with complete marginal integration (or with less than 1 mm demarcation border), and the defect site contained only few, scattered fissures.

4.4. Implantation of multiply incised, surface-enhanced cartilage allograft

The multiply-incised, lamellated chondral allografts were assessed 6 and 24 weeks following the implantation with macroscopic and microscopic evaluation criteria. After 6 weeks, the incised chondrografts showed stable osteochondral integrity without any sign of delamination, however, the marginal integration was lacking. After 24 weeks the appearance of the cartilage implants was highly satisfactory, receiving „Grade II: almost normal” mark with ICRS Macroscopic Assessment Score, but the circumferential cartilage integration was still incomplete. Microscopical analysis demonstrated that the osteochondral integration process was already started at 6 weeks postoperatively with subchondral irregularity. At the deep zone of cartilage graft calcification was present, and a slight cell invasion was detected within the previous incisions of the cartilage graft.

4.5. Comparative animal experiments with different cartilage repair methods

Porcine untreated cartilage defects without repair resulted in inferior quality tissue when assessed 24 weeks postoperatively. In the group treated with microfracture weak repair tissue appeared only at the margins of the defect. In the I. generation ACI group the macroscopic appearance of the repair tissue resembled hyaline cartilage, and the thickness and integrity of the repair was acceptable. The group treated with II. generation ACI demonstrated more even repair surface, with more precise marginal integrity, but with a few superficial fissures. The defects filled previously with multiply-incised chondrograft showed good-quality cartilage without any sign of delamination, but with incomplete marginal integration. The macroscopical comparison revealed statistically significant differences between the groups with no repair or microfracture, and the other three groups regarding the degree of defect repair and the macroscopic appearance.

In the groups with no repair or microfracture, osteochondral samples showed disorganized matrix and cell distribution typical of fibrocartilage by histological evaluation. In the case of microfracture, the subchondral bone occasionally indicated the original drill holes. The groups treated with ACI methods demonstrated repair tissue with hyaline characteristics, but the distribution pattern of chondrocytes was

distinguishable from the surrounding healthy cartilage tissue. Even macroscopically integrated cases showed minor gaps between the intact cartilage and the graft tissue. In cases with multiply-incised chondrograft we found hyaline cartilage with full osteochondral integration. The former cartilage cuts were no longer visible, but the marginal integration was incomplete, and the subchondral bone appeared irregular with less osseal trabecula. When the groups were compared, a strong statistical difference was found in two categories: the extracellular matrix and the cell distribution were significantly better in the ACI and incised chondrograft groups compared with the groups with no repair or with microfracture.

4.6. Introduction of autologous chondrocyte implantation method in human

After the ACI intervention we examined functional tests according to Lysholm-score in the affected knee joint. 6 months postoperatively the score values were increased compared to the preoperative status. The articular hydrops was retracted, the range of motion (ROM) was almost total, the patient did not complain about pain following minor strain. After 12 months, the functional tests showed further increase. By the 24 month-follow-up the deficiencies in loadability of the affected knee joint returned, therefore we got a lower score by the functional tests, but the increase remained significant compared to the preoperative status. The patient returned to active football, his previous joint effusions fully disappeared.

We evaluated the integration of the matrix with the help of MRI technique after 6 and 12 months. On the preoperative MRI pictures of the medial femur condyle we found abnormally thin and irregular cartilage layer, with oedema and softening of subchondral bone. The postoperative pictures demonstrated a normal thickness cartilage layer, and normal density of subchondral bony matrix.

5. Discussion

5.1. Chondrocyte tissue engineering in monolayer and matrix environment

It is a well known fact, that the chondrocytes lose their typical phenotype, special morphological and functional properties during cell expansion with the increasing number of cell divisions. Their phenotype become more and more fibrocyte-like, and they dedifferentiate later into fibroblasts. This phenomenon is a remarkable limiting factor in tissue engineering procedures based on chondrocyte cell expansion. There are several opportunities to avoid or minimize the dedifferentiation, e.g. cell expansion under low oxygen-pressure, three-dimensional hydrogel culture media (agarose, collagen, alginate, fibrin), culture media supplemented with several growth factors, or chondrocyte seeding onto three-dimensional biomaterial matrices.

In our experiments we used special culture medium, supplemented with growth factors and autologous blood serum, which proved to create proper conditions in a reproducible way for the chondrocyte cell expansion. Our cell cultivation method resulted in suitable number of chondrocytes, which were capable of being replanted onto the chondral defects. It has become apparent from our studies that the microscopic appearance of monolayer-cultured chondrocytes became more and more similar to that of fibrocytes with increasing number of cell passages. Our present findings are in fully accordance with recent results of other investigations.

The chondrocyte dedifferentiation processes are also accompanied by fundamental changes in gene expression of cartilage specific markers. The expanded chondrocytes start to produce collagen type I. (COL1A1) and type III. instead of producing collagen type II. (COL2A1), which is characteristic for normal cartilage. Increased production of low molecular weight proteoglycans (e.g versican) instead of large molecular weight aggrecan is also noticeable.

In our PCR studies we have experienced a gradual and significant decrease in gene expression of COL2A1, COMP, and aggrecan markers, which was most significant after the fourth passage of the chondrocytes. This dedifferentiation pattern corresponds with parallel results reported in the literature.

In our investigations we applied PGA-based three-dimensional matrix as a carrier of chondrocytes. According to our microscopical analysis the chondrocytes

showed good affinity to the filaments of matrix, the associative tendency was fairly observable. The chondrocytes were present in native distribution, since the cells were scattered through three different dimensions of the space.

Our gene expression analysis revealed an increased expression activity of COL2A1, COMP, and aggrecan markers after chondrocyte-seeding onto the matrix, with parallel decrease of expression of collagen type I., which is a dedifferentiation marker for chondrocytes and widely known to be attributable to fibrocytes. The mechanical stability of our matrix was sufficient, and its structure allowed to make stable fixation with intact cartilage surrounding the focal chondral defect.

5.2. Chondrocyte viability tests with steroids and local anesthetics

The intraarticularly administered corticosteroids and local anesthetics play an important role in the pharmacologic treatment of osteoarthritis. The corticosteroids proved to be efficient antiinflammatory agents with relative slow pain-relieving effect, contrarily, the local anesthetics show faster onset. The rationale of the combined use of the two agents would be that the local anesthetic component acts quickly after administration, it provides fast alleviation of pain, and it may last for up to the point when the steroid component starts to exert its effect. Previous studies have proved that both local anesthetics and glucocorticoids have cytotoxic effects on various cell types. As a consequence, any intraarticular medication in the treatment of osteoarthritis causing cytotoxicity of chondrocytes eventually would accelerate osteoarthritis.

Our in vitro results revealed that the combination of certain local anesthetics and steroids has an unexpectedly high cytotoxic effect. The combination of betamethasone and bupivacaine or ropivacaine induced almost 100% cell death in cell cultures. When investigated in osteochondral explants, betamethasone-lidocaine and especially prednisolone-lidocaine combinations exerted a high percentage of cell death among chondrocytes, throughout the entire cartilage depth. The combined forms induced a considerably higher cytotoxicity rate in chondrocytes compared with the effects of anesthetics or steroids alone.

Our observations imply a synergistic rather than just an additive effect of the glucocorticoid and local anesthetic on cell death. This finding may suggest their questionable role in the treatment of osteoarthritis.

5.3. Possible treatments for replacement of focal cartilage defects

In our macroscopic assessment we found only a weak marginal tissue formation at the borders of original chondral defects in the group treated with microfracture. The histological analysis showed disorganized matrix and cell distribution in this group, and the whole repair tissue resembled to fibrocartilage, which may account for lower mechanical stability.

In our ACI animal experiments the primary chondral defects were covered with repair tissue in 80-85% (I. generation ACI), and in 90-95% (II. generation ACI). Although the repair tissue included several (I. generation ACI), or scattered (II. generation ACI) fissures, the ACI groups demonstrated repair tissue with hyaline characteristics. The pattern of the cells however was distinguishable from the surrounding healthy cartilage, suggesting only a hyaline-like appearance. Even macroscopically integrated cases showed minor gaps between the intact cartilage and the graft tissue, when assessed microscopically.

Since the most fundamental criteria for the cartilage repair tissue is the proper fixation on the bony surface of the chondral defects, theoretically, transplanting pure cartilage to the defect site may seem to be a very attractive solution. The osteochondral integration of any cartilage graft is paramount for the longevity of the repair tissue.

We hypothesized that the fusion of the cartilage to its bony base could be accelerated and augmented by cutting into the deep zones of cartilage grafts. The multiple incisions greatly increase the integrational surface, resulting in more secure fixation on the bony surface. A further advantage of multiple incisions through the cartilage is the enhancement of chondrocyte motility and therefore fostering the new repair tissue production.

In our study we observed that the multiply-incised chondrograft had the ability to attach to the subchondral bone on the site of chondral defects, resulting in good-quality cartilage tissue. Based on our animal experiments we found multiple incisions as a safe, noncomplicated, and highly efficient method with encouraging results, which may pose the opportunity of its human use.

5.4. Introduction of autologous chondrocyte implantation method in human

We managed to introduce a complicated tissue engineering technique with quality control methods in the Department of Orthopedics without any financial influences from external companies. We established the basic conditions for chondrocyte implantation in the Department of Orthopaedics with the contribution of Department of Immunology and Biotechnology at University of Pécs.

In the course of our human intervention we have replaced a large chondral defect in the knee joint of a sportsman with II. generation ACI method. The long-term follow-up examinations showed impressive results, since the patient did not complain about previous articular pain or swelling after the surgical intervention, moreover, he returned later to active sport.

Despite the promising clinical results of the ACI method, the more frequent occurrence of this method is probably not expected in Hungary for the present, because of high financial costs.

Although fairly expensive chondrocyte implantation techniques are currently available by some biotechnological companies, the chondrocyte cultivation and reimplantation within one department can take up the fight against other cartilage repair methods based on its economic features.

6. Conclusion and summary of new findings

- we managed to set up standard, reproducible circumstances in our cell expansion laboratory and PCR method proved to be reliable to examine the gene expression activity of cultured chondrocytes
- we have investigated the legal background and implantation criteria of ACI method in legal systems of different countries
- based on the legal investigation of chondrocyte cultivation, the author of the current thesis defended her complementary degree thesis in postgraduate legal studies at University Pécs, Faculty of Law (Title: Legal Relations of Tissue engineering Techniques)
- we established the hungarian professional guideline for ACI for the first time, which is indispensable for using this technique in humans
- our chondrocyte viability studies showed a synergistic effect between cytotoxicity of local anesthetics and steroids. These findings may raise the question regarding whether intraarticular administration of local anesthetic-steroid combinations is justified in the treatment of osteoarthritis.
- PGA matrix proved to be reliable to carry chondrocytes, to simulate the normal scattering pattern of chondrocytes, to help the differentiation, and production of cartilage extracellular matrix
- our porcine articular cartilage models proved to be stable base to investigate different cartilage repair techniques
- our animal experiences with ACI methods showed reassuring results for replacement of focal chondral defects
- we designed a special, patented cartilage harvester and cartilage incisor for surface-enhanced, processed, multiply-incised, lamellated chondrograft
- multiply-incised pure cartilage chondrografts showed promising results for replacement of chondral defects
- in our comparative animal experiments we found significantly superior differences in II. generation ACI method and surface-augmented chondrografts
- we have carried out II. generation human ACI intervention in Hungary for the first time, which suggested promising results by the long-term follow-up

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