Title: Autologous Chondrocyte Implantation to repair Knee Cartilage Injury: Ultrastructural Evaluation at 2 years and long Term Follow up including Muscle Strength Measurements

Løken, Sverre[1, 2]; Ludvigsen, Tom C.[1]; Høysveen, Turid[4]; Holm, Inger[5], Engebretsen, Lars[1, 2]; Reinholt, Finn P.[3]

1. Orthopaedic Centre, Ullevål University Hospital and Medical School, Oslo, Norway. 2. Oslo Sports Trauma Research Center, Norwegian University of Sport and Physical Education, Oslo, Norway. 3. Institute of Pathology and the Pathology Clinic, Rikshospitalet University Hospital, University of Oslo, Oslo, Norway. 4. Norwegian Sports Medicine Institute, Oslo, Norway. 5. Department of Rehabilitation, Rikshospitalet University Hospital, University of Oslo, Oslo, Norway.

Abstract

Autologous chondrocyte implantation usually results in improvement in clinical scores. However, long term isokinetic muscle strength measurements have not been reported. Biopsies from the repair tissue have shown variable proportions of hyaline-like cartilage.

In this study 21 consecutive patients were treated with autologous cartilage implantations in the knee. Mean size of the lesions was 5.5 cm^2. Follow up arthroscopy with biopsy was performed at 2 years in 19 patients. The biopsies were examined with both light microscopy and transmission electron microscopy techniques including immunogold analysis of collagen type 1. Patient function was evaluated with modified 10 point scales of the Cincinnati knee rating system obtained preoperatively and at 1 and 8.1 years. Isokinetic quadriceps and hamstrings muscle strength testing was performed at 1, 2 and 7.4 years.

Light microscopy and transmission electron microscopy both showed predominately fibrous cartilage. The immunogold analysis showed a high percentage of collagen type I. At 7.4 years the total work deficits compared to the contra-lateral leg for isokinetic extension were 19.1% and 11.4%, and for isokinetic flexion 11.8% and 8.5% for 60º/sec and 240º/sec,

respectively . Mean pain score improved from 4.3 preoperatively to 6.3 at one year (p=0.031) and 6.6 at 8.1 years (p=0.013) postoperatively. Overall health condition score improved from 4.1 preoperatively to 6.1 at one year (p=0.004) and 6.5 at 8.1 years (p=0.008). 3 patients later went through revision surgery with other resurfacing techniques and are considered failures. In summary the formation of fibrous cartilage following autologous chondrocyte implantation was confirmed by transmission electron microscopy with immunogold histochemistry. Although the functional scores were generally good, strength measurements demonstrated that the surgically treated leg remained significantly weaker.

Introduction

Injured articular cartilage has a poor capacity for spontaneous healing. The ability to repair injured cartilage has long been a challenging goal for basic scientists and orthopaedic surgeons. Focal cartilage and osteochondral injuries are common also in young people [2] and they may cause pain and limit daily activities, working ability, recreational activities and sports. Cartilage injuries generate large costs for the individual and for the society. [30]

The first clinical results from the repair of focal cartilage injuries using autologous chondrocyte implantation (ACI) in the human knee were published in 1994 [9] and led to renewed interest in research to repair or restore injured articular cartilage. Until July 2004 61 studies with 3987 surgeries had been published.[25] Several studies on the short term results of ACI in single series have been published.[9, 13, 15, 16, 33] Only a few long term studies have been published. [37-39] In general the study designs have been poor with only four randomized controlled trials (RCTs) available.[6, 21, 26, 27, 42] These RCTs compare ACI to other methods such as osteochondral plug transfer or bone marrow stimulating procedures, and they also include histological results. Generally the clinical results are promising with 80-90% excellent to good results in the single series studies. Many different functional scoring systems have been used. The results from RCTs vary [5, 6, 21, 26, 27, 42] and taking all these studies together no method has proven to be superior to others.

The histological results vary in different studies. Some studies show mainly hyaline cartilage, [9, 39] while others show predominately fibrous cartilage.[27] In a recently published RCT, histology following ACI with the use of characterized chondrocytes that expresses a marker profile (a gene score) predictive of the capacity to form hyaline-like cartilage scored better than following microfracture.[42] It has been shown that, when cultivated in monolayers as

in ACI, human chondrocytes dedifferentiate into a fibroblast-like state and lose their ability to syntesize collagen type II.[43] The chondrocytes may preserve much of their phenotype when cultivated in their own matrix [44] or in agarose gel.[7] Whether a redifferentiation of the chondrocytes occurs after ACI have been questioned. [22] The present study combines biopsy results (light microscopy and transmission electron microscopy) at 2 years with long term clinical results and muscular strength measurements.

Material and methods

Patients with a confirmed or suspected full thickness (Outerbridge 3-4[36]) focal cartilage injury in their knee and with major symptoms related to this were selected as candidates for ACI. The current report includes the patients treated with this method from 1997 until 1999 when a randomized controlled multi-center study was started.[26, 27] Mean age was 28 years (range 16-45 years). There were 9 females and 12 males. Preoperative clinical assessment followed the protocol from the cell laboratory (Genzyme (Boston, Massachusetts). According to the surgeons form all patients had normal clinical femoral-tibial alignment (between 5 and 10 degrees) and normal patellofemoral tracking. Except two patients who had ACL reconstruction together with the ACI, all patients had stable knees. Three patients had an ACL reconstruction less than one year prior to ACI. Patient data are summarized in Table 1. Previous surgery had been performed in the same knee in 19 patients with the total of 33 surgeries. Cartilage related procedures were performed in 19 of the previous surgeries, mostly related to OCD lesions. Other procedures had been performed in 14 of the previous surgeries. One patient had been treated with microfracture 17 months prior to ACI. None of the patients had been previously treated with ACI or osteochondral sylinder transfer. Three patients have been through revision surgery with different resurfacing techniques and are considered

failures. Follow up was done at 1, 2 and 8.1 years (86-115 months). Muscle strength testing was performed at 1, 2 and 7.4 years (86-103 months).

Surgical method: The surgical technique described by Brittberg et al[9] was used. The patients underwent arthroscopy, and if an Outerbridge[36] grade 3 or 4 focal cartilage defect suitable for repair and no other likely causes of the symptoms were found, 2-300mgs (2-3 slices) of cartilage were harvested from the proximal edge of the medial trochlea or from the medial edge of the lateral femoral condyle. The biopsy specimen was then placed in a sterile transport medium provided by Genzyme (Boston, Massachusetts) and was shipped by express air transport for cell culturing in their laboratory in Boston. Cell implantation was performed at average 72 days (range 29 – 318 days) after cartilage harvest: An arthrotomy was performed, and the defect was débrided to healthy surrounding cartilage. Periosteum was obtained from the proximal part of the tibia and was sutured to the rim of the débrided defect. Fibrin glue was used to form a watertight chamber. The cultured chondrocytes were then injected beneath the patch, and a final suture and fibrin sealant were placed at the injection site.

At follow up arthroscopy after two years the quality of the repair was evaluated according to the ICRS cartilage repair assessment score (maximum score 12) for macroscopic appearance of the cartilage surface.[10, 24] This scoring system included evaluation of defect fill, integration with surrounding cartilage, and surface appearance. Two-millimeter-diameter core-biopsy specimens were obtained from the treated defect and included both repair cartilage tissue and subchondral bone. Re-arthroscopies in addition to the planned follow up arthroscopy and other complications were recorded.

Rehabilitation: Phase 1 (0-6 weeks): Patients were hospitalized for four days after the cell implantation procedure. Continuous passive motion and partial weight-bearing with crutches were started on the first postoperative day. The patients then remained partially weight-bearing (20 kg) with crutches for eight weeks. Stationary bicycling was started as soon as possible. *Phase 2 (6 weeks-6 months):* Full weight-bearing was introduced between eight and twelve weeks postoperatively, depending on the patient's clinical status and function with the aim to achieve a normal gait pattern. Balance training and closed kinetic chain exercises were emphasized. No weights more than the bodyweight were allowed the first 6 months. *Phase 3 (6-12 months):* More loads were added to the exercises. Running and jumping exercises were started. Non-pivoting activities like cycling, swimming, walking in terrain and cross-country skiing were encouraged. Pivoting activities were not allowed the first year. Competitive sports were not recommended the first 12-18 months. Training under the guidance of a physiotherapist was conducted 2-3 times per week, but with additional exercises on their own. All patients living in the local area (n=14) were followed closely by the same physiotherapist with special interest in rehabilitation after cartilage repair (TH), the remaining were followed by different physiotherapists, but according to an identical rehabilitation protocol. We have no systematical record of the rehabilitation after one year.

Evaluation of clinical results: Baseline data and preoperative functional status was obtained using modified 10-point scales of the Cincinnati knee rating system. This is a modification provided by the cell laboratory (Genzyme®) and also used in other studies [11, 33]. The same form was used at 1 and 8.1 years for the comparison of results at different time points. Two of the three patients that were reoperated with other resurfacing techniques had an available score before failure and were included in the 8.1 year analysis with this score. Three patients are not included in the analysis of the final results: Two of them due to missing preoperative

data (one of them a failure). The third suffered a cerebral insult during the ACI operation and her overall general health has been markedly impaired after this episode. SF-36 score [48] (SF-36 Norwegian version 1.2) and a standard Cincinnati knee rating system[3] score was obtained after 7.4 years. Isokinetic quadriceps and hamstrings muscle strength was measured using a Cybex 6000 (Cybex, Division of Lumex, Inc., Ronkonkoma, New York) at 1, 2 and 7.4 years. Before testing, the patients warmed up on a stationary bike for 8 minutes. The test protocol consisted of 5 repetitions at an angular velocity of 60 deg/s (strength), followed by a 1-minute rest period, and then 30 repetitions at 240 deg/s (endurance). The parameter used for analysis was total work expressed as percentage of the contra-lateral leg.

Histological analysis: The specimens were primarily fixed in a mixture of 1 % paraformaldehyde and 0.5 % glutaraldehyde in 0.1 M phosphate buffer for 24 h, decalcified in 7% EDTA in 0.1 M phosphate buffer added 0.5 % paraformaldehyde for 7 days and subsequently prepared according to one of two separate protocols. Thus, each biopsy was divided into two halves by a longitudinal section. One half was further fixed in 2 % glutaraldehyde for 24 h and embedded in an epoxy resin according to a routine protocol[1], while the other half was low-temperature embedded in Lowicryl HM20 (Chemische Werke Lowi GmbH, Waldkraiburg, Germany).[40] Samples of the embedded tissue blocks from both procedures were then sectioned at 1μm and stained with toluidin-blue for qualitative and quantitative light microscopy. The histological evaluation was performed by by two of the authors; SL and an experienced pathologist (FPR) with special knowledge in morphological analysis of cartilage. The technically best sections were subjected to morphometry by point counting[17] . Using a computer program (Analysis Pro®, Olympus, Münster, Germany) a grid with 100 μm between test lines was superimposed to micrographs obtained by systematic random sampling (Figure 1). Test points overlaying an area with fibrous cartilage and hyaline-like cartilage, respectively, were counted and the proportion of hyaline-like cartilage

was calculated. Each specimen was counted twice, and the mean values for the two countings were used. Hyaline like cartilage was differentiated from fibrocartilage by a more homogeneous appearance of the matrix, and the round or oval shape of the cells, which often were surrounded by lacunae. Fibrocartilage had bundles of collagen fibers, lying in a random, irregular manner. The quality of the repair tissue was classified according to Knutsen et al [27] where hyaline like cartilage ≥ 60% was classified as group 1, Hyaline like cartilage ≥ 40% and < 60% as group 2, and hyaline cartilage < 40% as group 3. Inadequate biopsies or biopsies with no repair tissue were classified as group 4. Integration to underlying bone was also evaluated. Following the light microscopic analyses representative ultrathin sections of the epon-embedded material were evaluated by qualitative transmission electron microscopy (TEM) with focus on cell integrity, matrix organisation and occurrence of matrix vesicles as an indicator of undergone apoptosis. Both areas showing a fibrous and hyaline-like repair at light microscopy were examined. Furthermore, from 2 selected biopsies the low-temperature embedded samples were subjected to immunogold labeling according to our established protocol [40] using monoclonal antibodies to human collagen type I (MP Biomedicals, LLC, Solon, Ohio, USA).

Statistical analysis: SPSS statistical package version 15 (Chicago, Illinois, USA, 2006) was used for statistical analysis. Friedman test with a significance level of 0.05 was used to analyze differences between all three time points in the modified 10-point subscales and isokinetic strength .If significant differences were detected, Wilcoxon non parametric test for paired samples was used for a similar comparison between two separate time points. A Bonferroni correction was applied so that p-values < 0.017 (< 0.05/3) were regarded as significant in the paired comparison. To compare isokinetic strength between affected and

unaffected side a Wilcoxon non parametric test for paired samples was performed at each separate time point with a significant level of 0.05.

Results

Clinical outcome: Isokinetic strength measurements (Table 2) showed a highly significant difference between the surgically treated leg and the contra-lateral leg at all time points. There was a reduction in the side to side differences from 1 to 2 years for both flexion and extension at both velocities. However, the only statistical significant reduction was seen in extension total work at 60 deg/s ($p=0.006$). From 2 to 7.4 years there was no change in the side to side differences, and the deficits for extension were still 19.1% and 11.4%, and for flexion 11.8% and 8.5% for 60º/sec and 240º/sec, respectively . At 8.1 years there was a statistical significant improvement in mean pain score and overall health condition score from preoperative status. (Table 3). The overall health condition score had also improved significantly at one year. There were no significant change from preoperative scores in type of sport activity, activity level, knee swelling and partial or total knee collapse. None of the variables changed significantly from 1 to 8.1 years. The number of patients in the different levels and type of sports activity are shown in Table 4 (frequency of training) and Table 5 (type of sport). 13 patients were training regularly on a weekly basis at one year and 14 patients at 8.1 years. 13 patients were doing some kind of sports activity at one year and 17 patients at 8.1 years, and of these, five patients (both at one and 8.1 years) were participating in running or pivoting sports. The mean standard Cincinnati knee rating system score was 77.5 (SD 15, range 57-100) at 7.4 years. The SF 36 subscales scores at 7.4 years are shown in Figure 2. The patients tend to score lower for the physical subscales compared to a

Norwegian reference population (age 30-39)[31], but the number of patients are too small for statistical comparison.

3 patients have been through revision surgery with other methods and are considered failures: One patient was treated with an osteochondral cylinder transfer after 23 months after an acute loosening of the transplant from trochlea. One patient (age 44 at ACI) had a unicondylar knee prostheses implanted after 43 months and one patient was treated with microfracture technique after 22 months.

Arthroscopic evaluation at rearthroscopy (planned or due to symptoms): 14 patients underwent the planned re-arthroscopy after 2 years. 5 other patients had a re-arthroscopy before two years due to symptoms (3 of these became failures). The two remaining patients had their first re-arthroscopy after 3 and 5 years, thus within 6 years all patients had a re-arthroscopy. 4 patients have had a second re-arthroscopy after 5-10 years, and one of them has had a third re-arthroscopy. The indications for these re-arthroscopies were pain and/or mechanical symptoms.

Data were available from the first re-arthroscopy in 20 patients with data shown in Table 6. Median ICRS cartilage repair assessment score was 10.5 (range 0-12), with full coverage, complete integration with surrounding cartilage and a fibrillated surface in the majority of the lesions. Macroscopic hypertrophy of the tissue was seen in 14 lesions. Two of the three failure patients had a detached graft and one had exposed bone in the centre of the grafted area. At the second re-arthroscopy after 5-10 years in 4 patients there was still full coverage in 3 of 4 transplants and no graft hypertrophy.

Histological evaluation: 19 biopsies were available for analysis. Data are shown in Table 7 and histology of biopsies is shown in Figure 3. Three biopsies showed a high degree of normal hyaline cartilage with a sharp border towards fibrous tissue. According to the surgical report these had been obtained from the border between the repair tissue and the surrounding cartilage. We believe that the hyaline cartilage in these biopsies is the original surrounding cartilage, so consequently these were also classified as Knutsen group 3. The median percentage of hyaline like cartilage in biopsies that were from the repair tissue only was 11.8 % (range 0-39.9%). All the TEM examinations showed an abnormal repair tissue with enlarged disorganized fibers running in all directions and massive cell death (Figure 4A-D). This was also the findings when hyaline like areas at toluidin-blue staining were analyzed with electron microscopy. TEM with immunogold analysis of two selected samples (Figure 5A-B) confirmed these findings with high staining for collagen type I.

Discussion

The principal finding of this study was that first generation ACI resulted in the formation of fibrocartilage evaluated by transmission electron microscopy also including immune histochemistry. Secondly, the study confirmed that despite an improved functional score, the knee function was seriously affected, as muscle strength was still markedly impaired after 7.4 years.

Our study population is a mixture of traumatic lesions and sequelea after osteochondritis dissecans. Other studies have shown that patients with defects following osteochondritis dissecans obtain similar clinical results as patients with traumatic lesions [37]. We have therefore, as in other studies, [26, 27] chosen to view these as one comparable group of patients.

The clinical results are similar to those presented by others using the same modified 10-point scales of the Cincinnati knee rating system.[11, 33] A weakness of our study is the use of this non validated score which has not been widely used. However, the score reports on the same important variables (pain, locking, knee collapse and swelling) as the Cincinnati knee rating system and other scoring systems. The Cincinnati score[5, 6, 28] and the SF 36 score[4] after 7.4 years are in the same range as in other studies.

Strength measurements after ACI have to our knowledge not been published. Cybex testing has been used in patients who have undergone ACL reconstruction[41] and knee arthroplasty.[8] Our group of patients demonstrates lower extension and flexion total work (expressed as percentage of contra-lateral leg) compared to ACL reconstructed patients and similar extension total work and lower flexion total work compared to knee arthroplasty patients in these studies.[8, 41] This indicates that ACI patients suffer marked functional impairment. In a group of healthy female handball players a significant side to side difference (5.5%) was found for flexion total work at 240 deg/s, with no difference in the other measurements.[20] The side to side differences in the current study are more pronounced and can therefore not be explained by normal side to side variation. It is important to notice that quadriceps force is the strength parameter mostly affected at all time points. Shelbourne et al [46] claim that restoration of muscle strength with the emphasis on quadriceps strength may relieve symptoms in patients with a so called de-conditioned knee, and that failure in regaining strength may contribute to the de-conditioning. The importance of quadriceps strength in knee function has also been shown in a recent study: Reduced preoperative quadriceps strength in ACL reconstructed patients was associated with poorer functional outcome and persistent quadriceps weakness after two years[14]. This may also be the case in

the current study, as most patients had suffered from chronic knee pain for years. We do not have detailed data of the training load and intensity over the years after ACI. However, the majority of the patients were followed closely by a physiotherapist during the whole follow up period and we have information about frequency of training and sports activity level (Tables 4 and 5). Most patients were training at a regular basis indicating that the patients worked to maintain their muscular strength, but obviously this effort was not sufficient to normalize the strength deficit. Unfortunately we do not have preoperative tests, so we cannot say to what extent this impairment is a result of the cartilage injury itself, of the surgery, or both. Patients with a traumatic injury and patients with OCD did not seem to be different in this respect, but the numbers were too small for comparison. However, the majority of the patients had long standing symptoms before surgery. In this situation it might be more difficult to regain normal muscle strength.

The proportions of hyaline like cartilage and fibrocartilage vary between studies. Different definition of hyaline like and fibrous cartilage and different ways of grouping the results make comparison difficult. Some authors find that the majority of the patients have a hyaline or hyaline like repair tissue (some include mixed hyaline/fibrous repair tissue in this group),[9, 18, 34, 39] some find that about 50% of the patients have hyaline or hyaline like repair tissue,[27, 29] while others like in the current study find that the majority have fibrocartilage or fibrous repair tissue.[45, 47] It has been common to use a qualitative histological grading system to evaluate cartilage biopsies. However the validity and reproducibility of such scores have been poor.[23] We therefore chose a more direct approach in the current study including a quantitative morphometric measurement to calculate the percentage of fibrous cartilage. With this method the investigator will have to define the character of the tissue for every crossing in the grid overlying the specimen, in contrast to other studies where the proportion

of fibrous cartilage has been estimated by an overall judgment. This quantitative method could possibly partly explain the high percentage of fibrous cartilage in this study. Different ways to evaluate and classify biopsies may also explain variation in results between studies. There are, of course, also probably real differences between series. This may be due to different cell handling technique from cartilage harvest to implantation, differences in the surgical techniques or rehabilitation, or different patient selection.

Several biopsy studies after first generation ACI have demonstrated a more hyaline like repair tissue in the deeper layers and a more fibrous tissue with remnants of the periosteum flap in the superficial layers.[9, 19, 39]. In the current study this was not a consistent finding. Hyaline like tissue could be found as islands both in the deep and the middle layer and most of the tissue integrating with bone had a fibrous appearance. This may partly be explained by the generally low proportion of hyaline like cartilage in our specimens. The patients in the current study were operated at an early stage of this surgical technique, and the procedure included a cross Atlantic two way transport of cartilage and cells. Today shorter transport from laboratory to the hospital is possible, cell culture techniques have been further developed[42] and the implantation of biomaterials seeded with cells have been introduced.[5, 32] . Clinical biopsy studies on third generation ACI with the use of cells seeded in a scaffold are limited, and clinical studies comparing first generation ACI with the use of periosteal flap to later generations ACI have not been conducted. ACI with a collagen cover (ACI-C) was compared to ACI with cells seeded in a collagen matrix (MACI) in an RCT, and showed fibrous tissue in the majority of the patients with no difference between the groups[5]. In 63 patients treated with arthroscopic implantation of chondrocytes in a hyaluronan scaffold (Hyalograft-C®) biopsies showed 56% fibrocartilage in biopsies taken before 18 months post implantation, 27% fibrocartilage in biopsies taken later than 18 months, and no fibrocartilage in biopsies from asymptomatic patients with biopsies taken later than 18 months[12].

TEM gives a more detailed picture of the tissue and additional information compared to light microscopy. We are not aware of other studies that have used TEM in the evaluation of the results of autologous chondrocyte implantation. Scanning electron microscopy (SEM) , used to evaluate the surface structure, have been used to study biopsies following ACI in clinical studies [21, 49] and in experimental studies.[35] Our focus was to use TEM to characterize sections of the biopsies to delineate extracellular matrix organization with particular attention paid on the fibrous elements including immunogold technique for demonstration of collagen I fibers. Our TEM results indicate that it is probably not possible to obtain normal cartilage in the attempt to repair a cartilage defect with first generation ACI.

Other weaknesses of the study are the limited number of patients and the lack of a control group. However, in contrast to most other studies that have included biopsies, the present study has nearly a complete set of biopsies from a consecutive group of 21 patients. The results from the muscle strength measurements are also useful without a control group due to the paired situation with the contralateral leg as a control.

Conclusion

In this study we found that first generation ACI yielded fibrous cartilage and the surgically treated leg remained markedly weaker compared the contra-lateral leg 7-8 years after surgery. Future studies will show if better techniques in cell culture, surgery and rehabilitation can improve the results. Muscle strength measurements may be useful in the clinical evaluation.

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Table 1 Baseline characteristics of the patients

Number of patients		21
Age, years		
	Mean (range)	28 (16 - 45)
Gender		
	Females	9
	Males	12
Etiology		
	Osteochondrotis dissecans	13
	Trauma	8
Number of lesions		
	1 lesions, no of patients	20
	2 lesions, no of patients	1
Site of lesion		
	Medial femoral condyle	16
	Lateral femoral condyle	3
	Trochlea	2
	Patella	1
Size of lesion (total area per patient)		
	Mean (range)	5.5cm^2 (2 - 15)
	≥2 and < 4 cm^2	6
	≥4 and < 6 cm^2	7
	≥ 6 cm^2	8
Concomitant surgery		
	ACL reconstruction, no of patients	2
Number of previous knee surgeries		
	0	2
	1	11
	≥2	8
Type of previous surgeries		
	Cartilage related procedures	
	Microfracture	1
	Fixation of OCD fragment	2
	Removal of osteochondral/OCD fragment	13
	Drilling of OCD	1
	Debridement of cartilage/OCD sequelae	2
	Total number of cartilage related procedures	19
	Other procedures	
	Diagnostic arthroscopy	2
	Meniscal resection	9
	ACL-reconstruction	3
	Total number of other procedures	14
	Total number of previous surgeries	33

Table 2 Muscle Strength measurements expressed as total work in Nm (mean [SD]). ACI treated leg in percentage of contra-lateral leg (%). P-values refer to side-to-side differences at each time point (Wilcoxon non-parametric test). Friedman non-parametric test used to test over-all difference in percentage of contra-lateral leg between the three time-points.
* Significant increase from 1 to 2 years (p=0.006)

	1 year (n=12)				2 years (n=12)				7.4 years (n=19)				
	ACI treated leg	Contra-lateral leg	%	p-value	ACI treated leg	Contra-lateral leg	%	p-value	ACI treated leg	Contra-lateral leg	%	p-value	p-value Friedman
Extension total work 60 deg/sec	657(171)	934(206)	70.3	0.003	769(174)	959(167)	80.2*	0.003	815(183)	1005(186)	81.1	0.001	0.045
Extension total work 240 deg/sec	1716(363)	2219(542)	77.3	0.006	1986(372)	2475(548)	80.2	0.008	2130(602)	245(615)	86.9	0.002	0.905
Flexion total work 60 deg/sec	481(129)	586(169)	82.1	0.002	541(130)	604(102)	89.6	0.015	537(171)	611(159)	88.2	0.002	0.497
Flexion total work 240 deg/sec	1216(387)	1480(379)	82.2	0.003	1370(244)	1582(330)	86.6	0.005	1359(464)	1536(545)	88.5	0.01	0.301

Table 3 Modified 10 point scales of the Cincinnati knee rating system (mean [SD]). Friedman non parametric test used to analyze differences between all three time points. If significant differences were detected ($p<0.05$), Wilcoxon non parametric test for paired samples was used for comparison between two separate time points, with p-values < 0.017 (Bonferroni-correction) regarded as significant.

				Friedman	Wilcoxon		
	preoper	1 year	8 years	p-value	p-value 0-1yrs	p-value 0-8yrs	p-value 1-8yrs
Sports activity level							
1-4, 1= highest level	2.56 (1.15)	2.35 (0.86)	2.10 (0.94)	0.328			
Type of sports activity							
1-6, 6 most demanding	3.79 (1.62)	3.89 (1.32)	4.21 (0.92)	0.922			
Knee pain							
0-10, 10 = no pain	4.32 (2.77)	6.33 (2.77)	6.63 (2.31)	0.009	0.031	0.013	0.194
Knee swelling							
0-10, 10= no swelling	6.53 (2.48)	8.44 (2.33)	8.42 (1.95)	0.046	0.043	0.062	0.335
Partially knee collapse							
0-10, 10 = no partial collapse	6.11 (2.62)	7.00 (3.31)	8.11 (2.05)	0.053			
Total knee collapse							
0-10, 10= no total collapse	8.10 (2.26)	8.44 (2.79)	8.95 (1.93)	0.150			
Overall health condition							
0-10, 10 = best	4.11 (1.86)	6.06 (2.69)	6.44 (2.28)	0.003	0.004	0.008	0.397

Table 4 Sports participations (numbers of patients at each level)

	Preop	1 year	8 years
Level 1 training 4-7 times per week	4	1	5
Level 2 training 1-3 times per week	5	12	9
Level 3 training 1-3 times per month	4	1	3
Level 4 no sports	5	3	2
Not stated	3	4	2

Table 5 Type of sports activity (numbers of patients at each level)

	Preop	1 year	8 years
Level 6 - High demand pivoting sports (e.g basketball, football)	4	2	2
Level 5 - Running and less demanding pivoting sports (e.g. tennis, skiing)	2	3	3
Level 4 - No running or pivoting (e.g. cycling, swimming)	6	8	12
Level 3 - Activities of daily life without problems- no sports	1	2	1
Level 2 - Activities of daily life with moderate problems- no sports	5	2	1
Level 1 - Major problems with activites of daily life - no sports	1	1	0
Not stated	2	3	2

Table 6 ICRS cartilage repair assessment score.[10, 24]

ICRS total score, median: 10.5 points (range 0 - 12 points)	Number of patients
Filling	
In level with surrounding cartilage - 4 points	15
75% repair of the defect - 3 points	2
50% repair of the defect - 2 points	0
25% repair of the defect - 1 point	1
0% repair of the defect - 0 points	2
Integration to border zone	
Complete integration with surrounding cartilage - 4 points	12
Demarcating border < 1 mm - 3 points	5
3/4 of graft integrated, 1/4 with a notable border > 1 mm width - 2 points	1
1/2 of graft integrated with surrounding cartilage, 1/2 with a notable border > 1 mm 1 point	0
From no contact to 1/4 of graft integrated with surrounding cartilage - 0 points	2
Macroscopic appearance	
Intact smoth surface - 4 points	1
Fibrillated surface - 3 points	14
Small, scattered fissured or cracks - 2 points	2
Several, small or few but lagre fissures - 1 point	1
Total degeneration of grafted area - 0 points	2

Table 7 Histology evaluation: Percentage hyaline like (HL) repair tissue calculated by point counting. Results given as percentage HL repair and classified in groups according to Knutsen et al.[27]

Histology - percentage hyaline-like repair, median: 11.8 % (range 0-39.9 %)	Number of patients
Knutsen group	
Group 1 (≥ 60% HL)	0
Group 2 (≥ 40% and < 60% HL)	0
Group 3 (< 40% HL)	18
Group 4 (no repair tissue)	1

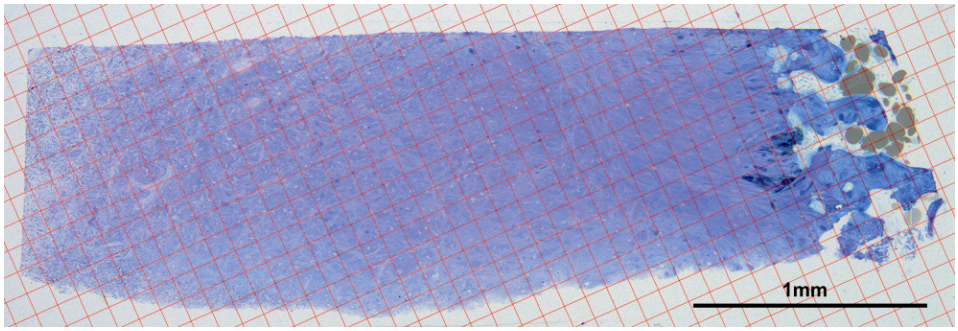

Figure 1. A square grid with 100µm between testlines superimposed to a micrograph to quantify hyaline-like and fibrous cartilage, respectively.

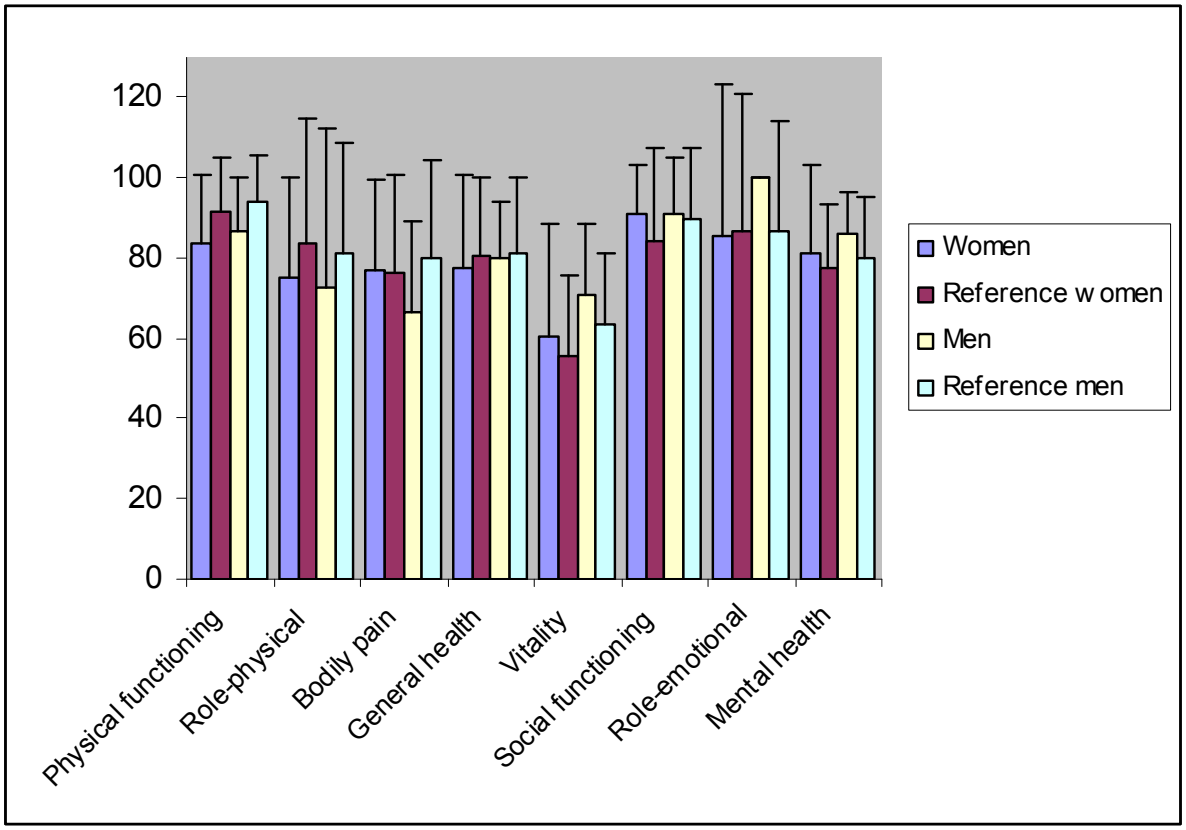

Figure 2. SF-36 subscales compared to reference values (age 30-39) from the Norwegian population.[30] Standard deviation showed as bars.

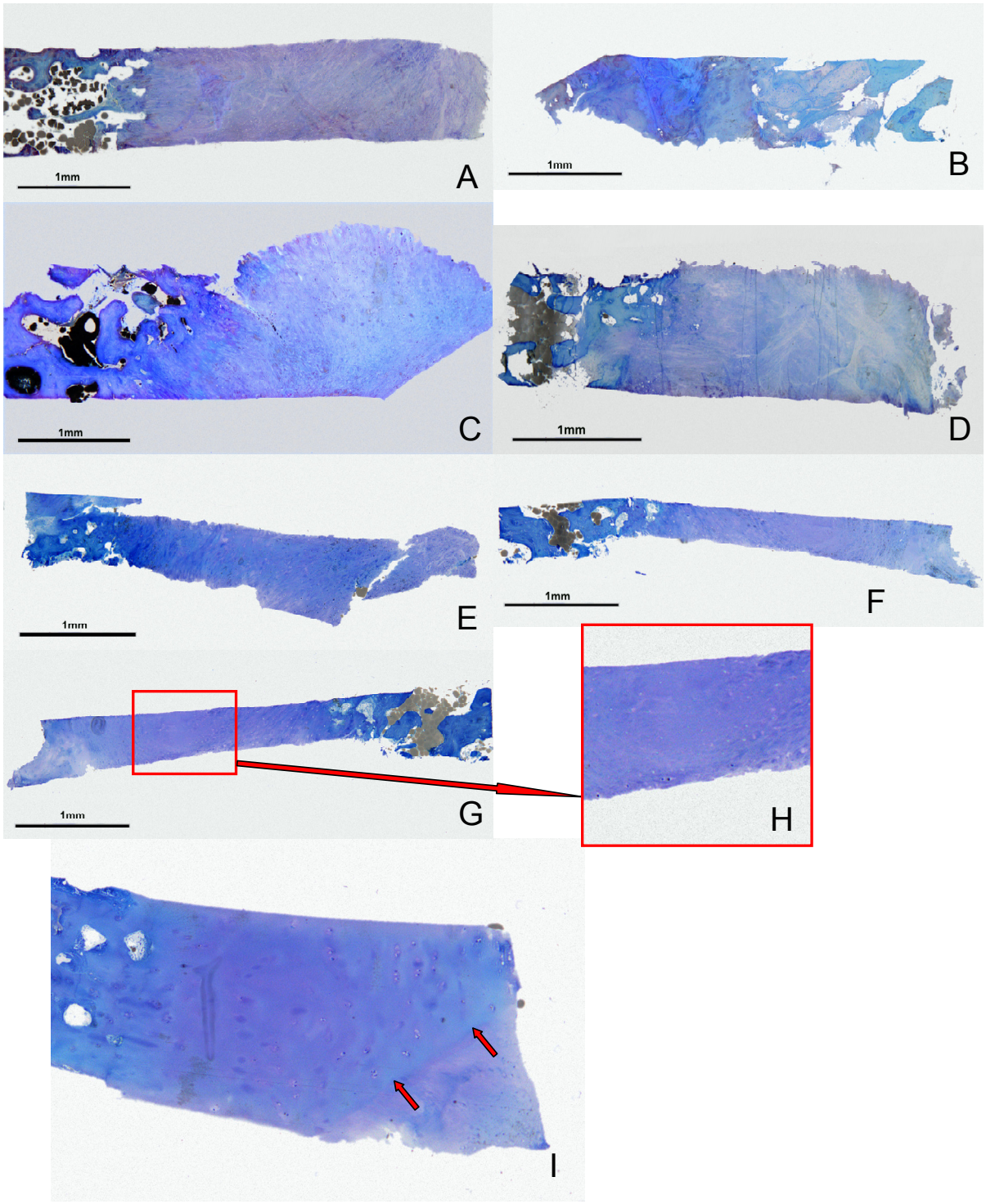

Figure 3. Biopsies of cartilage repair tissue stained with toluidine blue. Biopsies with mainly fibrocartilage (A-E). Biopsies with limited areas with hyaline-like repair (F-G). Magnified area with hyaline like repair (H). Biopsy taken from borderzone (red arrows) between graft and surrounding normal cartilage (I).

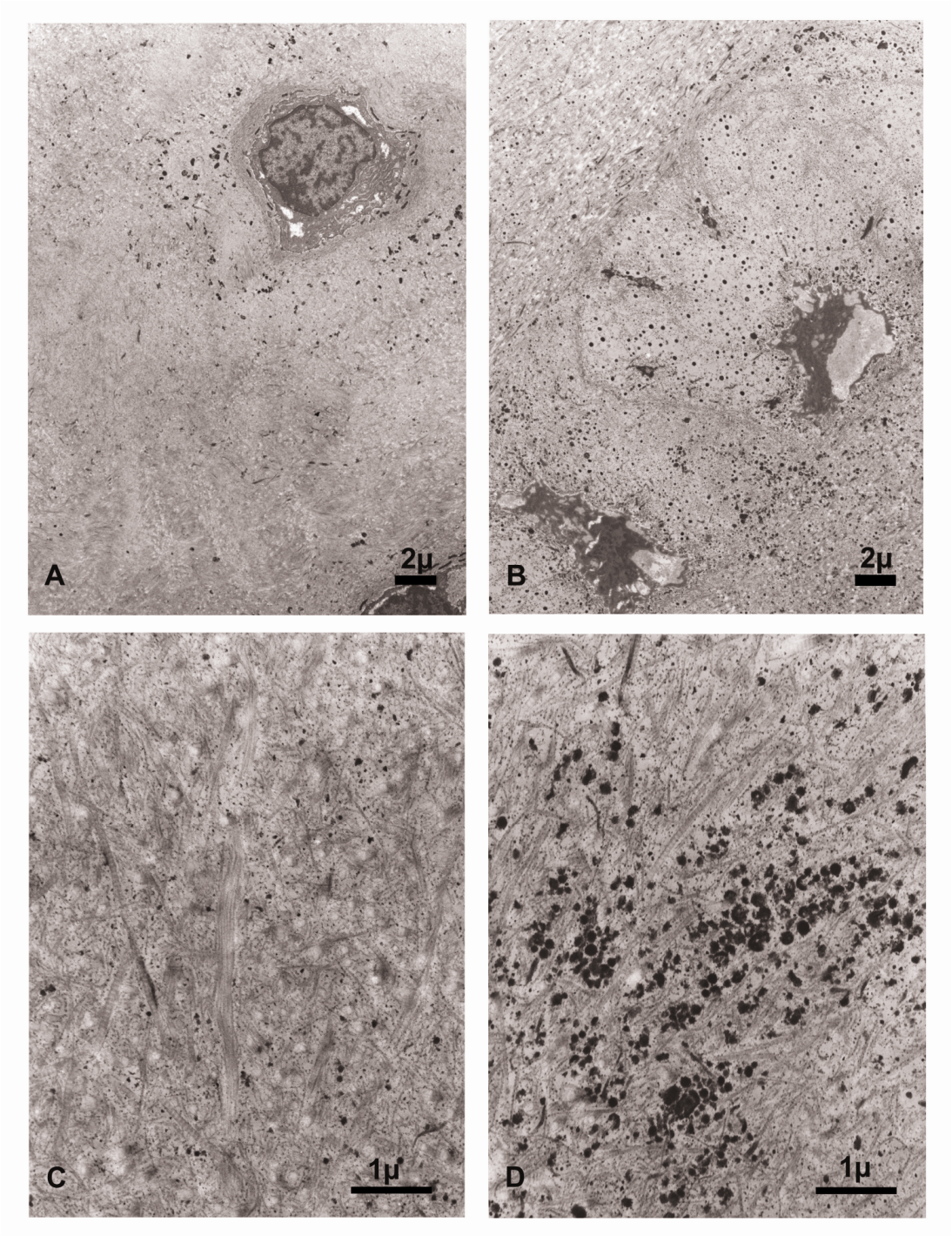

Figure 4. Low power electron micrograph from hyaline-like cartilage with a viable chondrocyte of normal ultrastructure (A). Low power electron micrograph from hyaline-like cartilage with chondrocytes in apoptosis (B). Medium power electron micrograph from hyaline-like cartilage showing increased variation in collagen fiber diameter with loss of orientation and crossing fibers (C). Medium power electron micrograph from hyaline-like cartilage showing an abundance of cellular debris, so-called matrix vesicles in the matrix (D). Epoxy resin embedded biopsies (Agar 100).

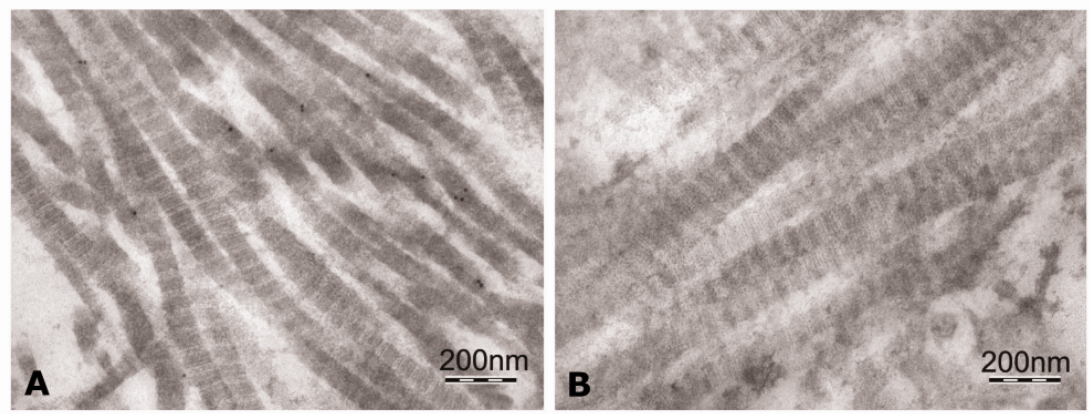

Figure 5. High power electron micrograph with immunogold labeling using antibodies to collagen I and protein A conjugated with 10 nm gold particles for detection. The black dots are gold particles (A). Control electron micrograph of the same area as in A of a section incubated with non-specific serum instead of antibody. The area is without immunogold labeling (B). Low temperature embedded biopsy (Lowicryl HM 20).