Nasal chondrocyte-based engineered autologous cartilage tissue for repair of articular cartilage defects: an observational first-in-human trial

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Summary

Background **Articular cartilage injuries have poor repair capacity, leading to progressive joint damage, and cannot be restored predictably by either conventional treatments or advanced therapies based on implantation of articular chondrocytes. Compared with articular chondrocytes, chondrocytes derived from the nasal septum have superior and more reproducible capacity to generate hyaline-like cartilage tissues, with the plasticity to adapt to a joint environment. We aimed to assess whether engineered autologous nasal chondrocyte-based cartilage grafts allow safe and functional restoration of knee cartilage defects.**

Methods **In a first-in-human trial, ten patients with symptomatic, post-traumatic, full-thickness cartilage lesions (2–6 cm²) on the femoral condyle or trochlea were treated at University Hospital Basel in Switzerland. Chondrocytes isolated from a 6 mm nasal septum biopsy specimen were expanded and cultured onto collagen membranes to engineer cartilage grafts (30×40×2 mm). The engineered tissues were implanted into the femoral defects via mini-arthrotomy and assessed up to 24 months after surgery. Primary outcomes were feasibility and safety of the procedure. Secondary outcomes included self-assessed clinical scores and MRI-based estimation of morphological and compositional quality of the repair tissue. This study is registered with ClinicalTrials.gov, number NCT01605201. The study is ongoing, with an approved extension to 25 patients.**

Findings **For every patient, it was feasible to manufacture cartilaginous grafts with nasal chondrocytes embedded in an extracellular matrix rich in glycosaminoglycan and type II collagen. Engineered tissues were stable through handling with forceps and could be secured in the injured joints. No adverse reactions were recorded and self-assessed clinical scores for pain, knee function, and quality of life were improved significantly from before surgery to 24 months after surgery. Radiological assessments indicated variable degrees of defect filling and development of repair tissue approaching the composition of native cartilage.**

Interpretation **Hyaline-like cartilage tissues, engineered from autologous nasal chondrocytes, can be used clinically for repair of articular cartilage defects in the knee. Future studies are warranted to assess efficacy in large controlled trials and to investigate an extension of indications to early degenerative states or to other joints.**

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Introduction

Articular cartilage injuries remain a clinical challenge and are associated with pain, disturbed function, and disability. About 2 million patients are diagnosed with articular cartilage defects every year in Europe and the USA.[1] When not treated, such lesions predispose to osteoarthritis and might result in total replacement of the joint, with limits of implementation in younger individuals and massive costs for the health-care system.[2] Cartilage repair treatments have the potential not only to relieve pain and improve the quality of life for younger patients but also to delay or eliminate the need for joint replacement. Current therapeutic options—eg, arthroscopic debridement, microfracture, autologous osteochondral grafting, and use of allografts or platelet-rich plasma—have major drawbacks, such as applicability to limited size defects, long and complex rehabilitation times, donor-site morbidity, or graft material availability.[3] Even advanced therapies based on autologous articular chondrocyte implantation, although improving symptoms in short-term follow-up, cannot reproducibly and durably restore cartilage structure and function, and have yet to prove cost effective.[4] Use of an autologous cell source with superior and less donor-dependent cartilage-forming capacity might enhance regenerative processes and lead to a predictable benefit for individual patients.

Chondrocytes from the nasal septum, compared with those from articular cartilage, show superior and more reproducible chondrogenic capacity, even across individuals of different ages.[5–7] The chondrogenic properties of nasal chondrocytes are maintained after extensive culture expansion, so that a small biopsy specimen, obtained under minimally invasive conditions and with no relevant discomfort, is sufficient to generate biochemically and biomechanically mature grafts of clinically relevant dimensions.[8] Indeed, engineered

Research in context

Evidence before this study

We searched MEDLINE for reports published in any language up to April, 2016, with the terms "nasal chondrocytes" and "articular cartilage repair". We identified 16 publications, of which 14 were related to in-vitro experiments and two were reporting in-vivo tests in a rabbit or goat model. In the study with the goat model, implantation of nasal chondrocytes in human articular cartilage lesions was reported to be part of an ongoing clinical trial, which is now the subject of this report. Extending the second search term to "cartilage repair" beyond articulating joints identified one report of a completed clinical study, related to nasal lobule reconstruction after tumour resection.

Added value of this study

Our study shows the feasibility, safety, and preliminary evidence of clinical efficacy of engineered nasal cartilage grafts for post-traumatic articular cartilage injuries. Compared with conventional autologous articular chondrocyte implantation, the novelty of the described approach is related to use of cells derived from the nasal septum, which display superior and less donor-dependent chondrogenic capacity, and implantation of a developed cartilage tissue versus undifferentiated cells delivered as a suspension or through a scaffold (effectively, we tested a tissue therapy instead of a cellular therapy). The possibility to obtain a nasal cartilage biopsy specimen under minimally invasive conditions, by contrast with the need for arthroscopy for articular chondrocytes isolation, is an added benefit for the proposed treatment.

Implications of all the available evidence

Beyond self-assessed patients' satisfaction, we established temporal maturation of repair tissue, approaching the composition of native hyaline cartilage. This achievement could be related to the chondrogenic capacity of the delivered nasal chondrocytes or the presence of mature cartilaginous extracellular matrix around them, or both. Further randomised trials comparing our approach with conventional treatments are needed to provide definitive data for the efficacy of the grafts. Demonstration of a positive effect of this procedure on the reproducibility and durability of repair might produce a major shift in the treatment of challenging cartilage lesions, for which no current treatment is yet satisfactory.

grafts based on autologous nasal chondrocytes have been used as an alternative to native cartilage for the reconstruction of the alar lobule of the nose after skin tumour resection, leading to complete structural, functional, and aesthetic recovery.[9]

The compatibility of grafts derived from nasal chondrocytes with implantation at an articular cartilage injury site is supported by findings of previous studies. For example, in one study, nasal chondrocytes responded to physical forces resembling joint loading in a similar manner to articular chondrocytes and upregulated molecules typically involved in joint lubrication.[10] Moreover, nasal chondrocytes recovered after exposure to inflammatory factors typical of joint injuries[11] and led to formation of hyaline tissue in rabbit articular cartilage defects.[12] Furthermore, nasal chondrocytes could adopt the molecular identity of articular chondrocytes once implanted in a joint and contributed actively to repair of experimental goat cartilage defects.[13]

Our study aimed to assess the safety, feasibility, and potential efficacy of cartilage grafts engineered from autologous nasal chondrocytes for the treatment of post-traumatic cartilage injuries in the knee. The distinct innovation and potential advantage of our study relates not only to use of cells of superior and more reproducible chondrogenic capacity (nasal *vs* articular chondrocytes) but also—as a direct result of cellular quality—to implantation of tissues rich in hyaline-like extracellular matrix. This idea contrasts with typical use of suspensions of undifferentiated cells or of cell-seeded scaffolds not yet developed as mature cartilage tissues.

Methods

Study design and patients

We did an observational first-in-human study at University Hospital Basel in Switzerland. We enrolled patients aged 18–55 years old with post-traumatic full-thickness cartilage lesions (2–6 cm², International Cartilage Repair Society [ICRS] grade III or IV) on the femoral condyle or trochlea. Further inclusion and exclusion criteria are listed in the appendix (p 3).

We designed this study in accordance with the Declaration of Helsinki and it was approved by the ethics committee of Basel (EKBB 92/11) and the Swiss Agency for Therapeutic Products (Swissmedic, TpP-I-2012-001). The clinical trial followed European guidelines on Advanced Therapy Medicinal Products (ATMP), Good Clinical Practice (GCP) guidelines, and Swiss laws for transplant products. All patients gave written informed consent.

Procedures

For isolation of chondrocytes, plastic surgeons harvested autologous septal cartilage (6 mm diameter) under local anaesthesia via a Killian incision (about 10 mm cranial to the caudal border of the septum and about 5 mm dorsal to the transition zone to the praemaxilla) using a punch biopsy tool (figure 1A and 1B).[9] On the same day, we collected 72 mL of venous blood to prepare autologous serum. We transported the cartilage biopsy specimen and blood sample to the Good Manufacturing Practice (GMP) facility and processed them according to the defined standard operating procedures and the established quality management system, as required by

See Online for appendix

GMP guidelines. The manufacturing process and facility were approved by Swissmedic (manufacturing authorisation number 32330).

Graft manufacturing and sterility controls were done as described elsewhere.[9] Briefly, after the cartilage biopsy specimen was separated from the perichondrium, cut into small pieces (figure 2A), and digested enzymatically, we cultured isolated nasal chondrocytes for 2 weeks in medium supplemented with 5% autologous serum, 5 ng/mL fibroblast growth factor 2 (FGF2) and 1 ng/mL transforming growth factor β1 (TGFB1; R&D Systems, Minneapolis, MN, USA; figure 2B and 2C). Cells were counted manually by two operators according to a validated procedure, and we ascertained cell viability using 0·4% Trypan blue (Sigma Chemical, St Louis, MO, USA). We seeded expanded nasal chondrocytes on the porous side of a collagen type I/III membrane (Chondro-Gide; Geistlich Pharma AG, Wolhusen, Switzerland) at a density of 50 million cells per 30×40×2 mm membrane.[9] To support matrix deposition and assembly, we cultured nasal chondrocytes in the membrane for 2 weeks using medium supplemented with 5% autologous serum, 10 µg/mL insulin (Novo Nordisk, Bagsvaerd, Denmark), and 0·1 mmol/L ascorbic acid 2-phosphate (Sigma Chemical).

To comply with regulatory requirements, we assessed nasal chondrocytes from three donors not included in this cohort for potential in-vivo tumorigenesis (appendix p 1). We cultured expanded nasal chondrocytes for 2 days on a collagen type I/III membrane (10×10×2 mm; Chondro-Gide; Geistlich Pharma AG) under the same conditions as described for generation of the clinically used grafts and implanted them into subcutaneous pockets of 8-week-old mice (NOD.Cg-PrkdcscidIl2rgtm1Wjl/SzJ [NSG]; Charles River Laboratories, Kisslegg, Germany). Animal procedures were approved by the Swiss Federal Veterinary Office (Kantonal permit BS-2590). After 6 months, when tumorigenic cells lines typically develop tumours at the implantation site,[14,15] mice were euthanised with CO_2 and inspected for formation of tumours both in the constructs and in different organs (lung, liver, kidneys, spleen, and local lymph nodes). None of the mice showed weight loss or abnormalities by palpation. Implanted constructs consistently developed cartilaginous, tumour-free tissues (appendix p 1). All explanted organs appeared macroscopically normal and no evidence of tumour formation was observed histologically.

Throughout the graft manufacturing phase for the clinically used constructs, regular sterility controls were done, also considering the possibly greater risk for infection with biopsies obtained from the nasal septum compared with those from other typical harvest sites—eg, articular cartilage. For every patient, we did three BacT/ALERT anaerobic and aerobic tests (BioMérieux, Durham, NC, USA) in the microbiology laboratory at University Hospital Basel, after culture for 1 day, 14 days, and 25 days. Mycoplasma detection tests were done by Biolytix AG (Witterswil, Switzerland) after culture for 1 day, 14 days, and 21 days.

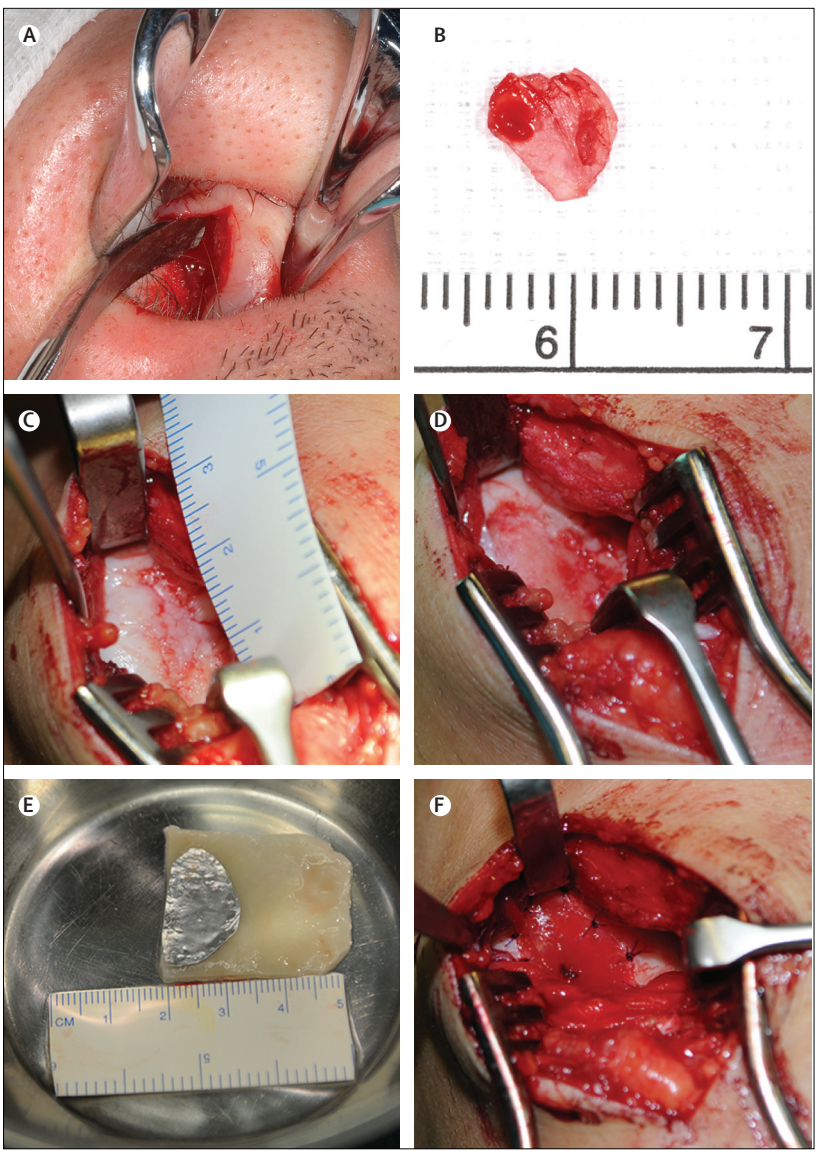
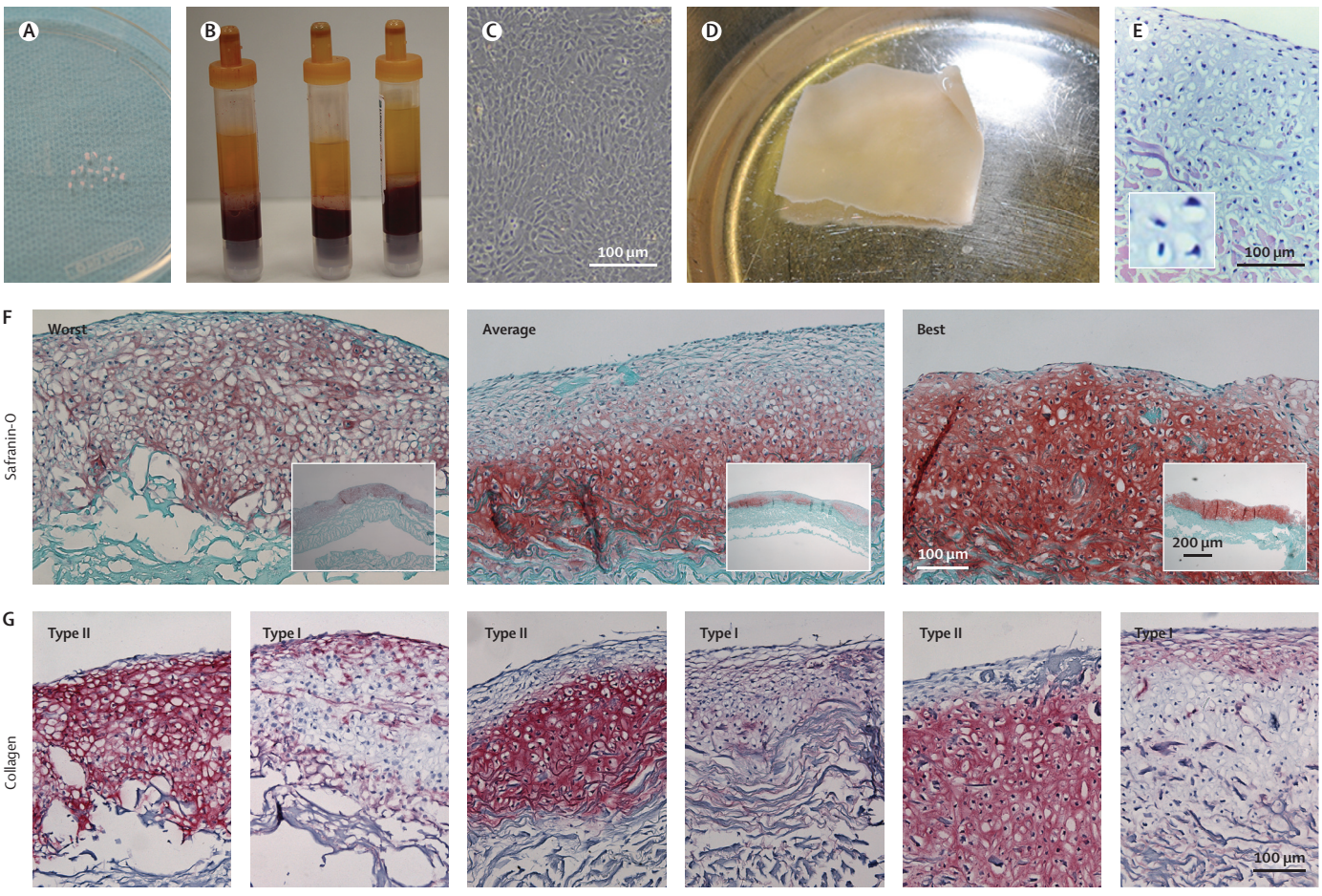


Figure 1: **Surgical procedure**
(A, B) Harvesting of a cartilage biopsy from the patient, under local anaesthesia. (C) Exposure of the full thickness cartilage defect of the lateral femoral condyle via mini-arthrotomy. (D) Refreshing of the cartilage lesion to remove the damaged cartilage. (E) Tissue engineered cartilage cut to the right shape and ready for implantation. (F) Tissue engineered cartilage inserted in the cartilage defect and secured by absorbable sutures.

Quality control tests for release of the grafts included absence of any contamination of cultured media, white and glossy visual appearance of the graft, structural stability through manual handling with forceps, and presence of at least 70% viable cells throughout the deposited extracellular matrix. We estimated the percentage of viable cells by frozen section analysis with haematoxylin and eosin staining of a strip (4 mm wide) resected from the margin of the engineered grafts within



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	Patient number									
	1	3	4	7	**8**	6	9	5	2	**10**
Mean µgGAG/µgDNA (SD)	**7·83 (2·06)**	8·04 (1·99)	8·07 (1·97)	9·15 (0·62)	**11·44 (2·61)**	12·56 (5·32)	13·39 (0·82)	16·57 (0·91)	18·86 (0·14)	**35·28 (3·57)**

Figure 2: **Generation and characterisation of the engineered cartilage graft**
(A) Macroscopic view of the biopsy of nasal cartilage septum after chopping. (B) Autologous blood after centrifugation. (C) Phase contrast images of confluent nasal chondrocytes. (D) White and glossy appearance of an engineered cartilage graft. (E) Haematoxylin and eosin staining of frozen sections to fulfil release criteria for graft implantation. (F) Safranin-O and (G) immunohistochemical staining for collagen types II and I of engineered grafts obtained for patient number 1 (worst, left), number 8 (average, centre), and number 10 (best, right). (H) Biochemical quantification of glycosaminoglycan (GAG) content, normalised to the DNA content of cartilage grafts.

20 min of tissue sampling. Based on these criteria, we released grafts and transported them to the operating room for implantation.

We did the operation by mini-arthrotomy. The cartilage defect was visualised (figure 1C) and debrided down to the subchondral bone and to healthy surrounding cartilage with a sharp spoon. After refreshing the cartilage lesion (figure 1D), the graft was trimmed to the individual defect size (figure 1E) and placed into the defect with the cell layer towards the exposed subchondral bone and the cell impermeable layer towards the joint space. The graft was then secured to the adjacent cartilage by absorbable sutures (Monocryl 5-0; Ethicon, Somerville, NJ, USA) and to the subchondral bone by fibrin glue (figure 1F). We adapted the rehabilitation programme from one described elsewhere,[16] with the main difference of immobilisation in extension in the first 2 weeks to reduce the risk of graft delamination.

We sent the non-used portion of the graft back to the tissue engineering laboratory at the Departments of Surgery and Biomedicine (University Hospital Basel), where it was cut into different parts for histological and biochemical assessments (at least two fragments per analysis). We fixed samples for histological analysis in 4% formalin, paraffin-embedded, and cross-sectioned (7 µm thick). We stained sections for sulphated glycosaminoglycans with safranin-O or processed them for immunohistochemistry using antibodies

	Patient 1	Patient 2	Patient 3	Patient 4	Patient 5	Patient 6	Patient 7	Patient 8	Patient 9	Patient 10
Gender	Male	Female	Male	Male	Male	Male	Male	Male	Female	Male
Age at implantation (years)	27	52	44	52	36	41	24	40	19	35
Body-mass index (kg/m^2)	23·0	21·6	30·3	24·7	25·4	26·9	27·7	28·4	28·7	27·2
ICRS defect grade	IV	IV	IV	IV (condyle); III (trochlea)	IV (condyle); III–IV (trochlea)	IV	IV	IV	IV	III
Defect size (cm^2)	3·8	2·0	2·0	2·4 (condyle); 1·0 (trochlea)	2·0 (condyle); 2·0 (trochlea)	4·5	2·7	5·0	6·0	2·0
Onset of symptoms	7 years	6 months	6 months	9 months	10 months	10 months	14 months	16 years	6 months	12 months
Previous surgical interventions (n)	2	1	0	0	0	1	1	1	1	1
Concomitant pathological findings	Meniscus tear	Meniscus tear	None	None	None	Meniscus tear, previous ACL repair	Meniscus tear	Meniscus tear, previous ACL repair	Arthrofibrosis, previous ACL repair	Meniscus tear
Locations	Femoral condyle	Femoral condyle	Femoral condyle	Femoral condyle and trochlea femoris	Femoral condyle and trochlea femoris	Trochlea femoris	Femoral condyle	Femoral condyle	Femoral condyle	Femoral condyle

ACL=anterior cruciate ligament. ICRS=International Cartilage Repair Society.

Table 1: **Patients' baseline characteristics**

against collagen type II (mouse anti-human, clone II-4CII; MP Biomedicals, Illkirch, France), type I (mouse anti-human I, clone I-8H5; MP Biomedicals), and type X (mouse anti-human, clone ab49945; Abcam, Cambridge, UK). Samples for biochemical analysis were digested with proteinase K; we quantified glycosaminoglycan and DNA content as described elsewhere.[11]

In one patient, a new cartilage lesion arose on a different part of the knee and reoperation was required; therefore, the patient was asked for consent to take a 2 mm punch biopsy at the original defect site during knee arthroscopy. We stained cross-sections of the biopsy specimen with safranin-O and antibodies against type I, type II, and type X collagens.

We assessed morphological and compositional characteristics of tissue at the repair site (hereafter referred to as repair tissue) in vivo using a 3T MRI scanner (MAGNETOM Verio, Siemens, Erlangen, Germany), 6 months and 24 months after surgery. We followed a standardised protocol for administration of contrast agent, acquisition of delayed enhanced images, and data analysis, as described elsewhere.[17] Images were analysed by consensus of a skilled musculoskeletal radiologist (MK) and an orthopaedic surgeon (MM), who were not masked, using an open-source DICOM viewer (OsiriX; Pixmeo SARL, Bernex, Switzerland). We graded morphological features of repair tissues with respect to their similarity to and congruity with the surrounding cartilage, with the Magnetic Resonance Observation of Cartilage Repair Tissue (MOCART) grading system.[18] We judged compositional quality of repair tissue in terms of glycosaminoglycan content, estimated by delayed gadolinium-enhanced MRI, and we measured water and collagen content, estimated by transverse relaxation time (T2). Contrast enhancement (ΔR1) is defined as the difference between the longitudinal relaxation rates (R1=1/T1) post-gadolinium and pre-gadolinium

	Patients (n)	Timepoint
Serious adverse events		
New cartilage lesions in the afflicted knee at other location, with admission and new surgery	1	12 months
New sports injury in the contralateral knee with admission and surgery	1	17 months
Adverse events		
Meniscus lesion of contralateral knee	1	11 months
Ankle distorsion	1	11 months
New sports injury of the afflicted knee without admission or surgery	2	20 months and 11 months

Adverse events and reactions recorded for nine patients who reached 24-month follow-up.

Table 2: **Adverse events and serious adverse events**

administration and is related inversely to the glycosaminoglycan content of tissue.[19] We used ΔR1 to calculate the relative ΔR1 (rΔR1), expressing the glycosaminoglycan content of the repair tissue by comparison with normal cartilage of the same joint (rΔR1>1 indicated a lower glycosaminoglycan content in the repair tissue and rΔR1<1 indicated a higher glycosaminoglycan content, compared with normal cartilage).[20] We estimated quantitative T2 times using a double-echo steady-state approach.[21]

We asked patients to assess their satisfaction with their knee before the procedure and 24 months afterwards using two self-assessment scores. First, we used the International Knee Documentation Committee (IKDC) subjective knee evaluation form; scores on this form range from 0 to 100, with 0 representing poorest knee function and 100 representing best knee function. Second, we asked patients to complete the five subscales of the Knee injury and Osteoarthritis Outcome Score (KOOS), to obtain their opinion on pain, other symptoms related to the knee, function in daily living, function in sport and recreation, and knee-related quality of life; scores on these subscales range from 0 to 100, with 0 representing poorest knee function and 100 representing best knee function.

Outcomes

The primary outcomes of the trial were feasibility and safety of the procedure. We defined feasibility based on achieving the quality control release criteria for the clinically used graft and suitability of the graft for intraoperative manipulation and implantation into the knee cartilage defect. We assessed safety up to 24 months after implantation of the graft into the knee cartilage defect, based on documentation of adverse events and their further classification into serious adverse events or serious adverse reactions, according to European Medicines Agency guidelines.[22] All adverse events, either local (eg, infection, haematoma) or systemic (eg, fever, allergic reaction), were handled according to GCP guidelines of the International Conference on Harmonization (ICH-GCP guideline E6 [R1]) and the Verordnung über klinische Versuche mit Heilmitteln (VKlin; Oct 17, 2001). Secondary outcomes were: patients' satisfaction by self-assessment, before surgery and 24 months after surgery; morphological quality of repair tissue 6 months and 24 months after surgery; and compositional quality of repair tissue 6 months and 24 months after surgery.

Statistical analysis

We assessed changes in clinical scores and radiologically measured cartilage properties at different follow-up times by paired *t* tests or Wilcoxon signed rank tests, according to the normality of the population; normality was assessed by D'Agostino-Pearson omnibus tests. We set significance at a two-tailed probability level of 0·05. We used SPSS version 22 for analyses.

Role of the funding source

The funder had no role in study design, data collection, data analysis, data interpretation, or writing of the report. The principal clinical investigators (MM, MJ) and scientific investigators (AB, IM) had full access to all data in the study and had final responsibility for the decision to submit for publication.

Results

Between Aug 29, 2012, and April 28, 2016, ten patients were enrolled into the study (two women and eight men). Patients' baseline characteristics are shown in table 1. Nine patients reached 24 months of follow-up. One individual was excluded because of independent sport injuries, which needed additional surgery at various sites, including the same location of the repair tissue. Two patients received treatment for two separate defects, using two fragments of the same engineered graft.

An adequate volume of autologous serum was obtained from all patients (mean 31·1 mL [SD 3·8]). After the expansion phase, all patients had sufficient cell numbers for manufacture of cartilage grafts (mean 98·0 million cells [SD 17·2], with mean 99·0% viability [SD 0·7]; figure 2C). No microbiological or mycoplasma contamination was noted on the control sterility tests, for any manufacturing batch. The cartilage grafts consistently had a white and glossy appearance (figure 2D). Frozen section analysis indicated that constructs contained at least 70% viable cells and abundant extracellular matrix (figure 2E). Thus, every manufactured graft fulfilled the defined quality control release criteria for implantation. In all cases, the manufactured grafts were large enough to cover the defect areas and were structurally stable. They could be trimmed and handled as required for mini-arthrotomy implantation and secured by sutures and fibrin glue, thus fulfilling the defined intraoperative manipulation and implantation criteria.

Histological and biochemical assessment of non-used portions of the engineered cartilage constructs showed intense staining for glycosaminoglycan, although with variable degrees of intensity and spatial uniformity (figure 2F). Additional immunohistochemical characterisation indicated the hyaline nature of the extracellular matrix: positivity for type II collagen was abundant in areas stained for glycosaminoglycan, whereas positivity for type I collagen was restricted mostly to the construct periphery (figure 2G). Staining for type X collagen, a marker of cartilage hypertrophy, was negative in all engineered grafts (data not shown). Biochemical analyses were consistent with the histological results: the amount of glycosaminoglycan and DNA was abundant in all constructs (mean 13·3 µg glycosaminoglycan/µg DNA [SD 9·5]), with an expected degree of variability among donors (figure 2H). These findings further confirmed the degree of maturation of the grafts as cartilaginous tissues.

No adverse reaction or adverse event occurred at the site of septum cartilage biopsy. No adverse reaction or serious adverse reaction was observed during 24 months after implantation of the graft into the knee cartilage defect. Two serious adverse events and four adverse events were recorded (table 2). One serious adverse event was caused by independent injury at another location (opposite knee), requiring orthopaedic surgery. The second serious adverse event was attributable to new cartilage lesions at other locations (ie, lateral femoral condyle and trochlea femoris) in the afflicted joint, which needed arthroscopic surgery. Expected adverse events (eg, postoperative pain and swelling) were not recorded as adverse events.

Mean IKDC scores and the five KOOS subscores all improved significantly from before surgery to 24 months after surgery (figure 3). Mean scores increased despite one patient being diagnosed with a new cartilage lesion in the same joint, with associated worsening of clinical status.

Morphologically, the level of repair tissue filling the defects was variable among patients and between the times of observation (figure 4A). Deterioration attributable to exposure of the subchondral bone in two defects, one of which occurred after a new sports injury (table 2), was mirrored by a significant decrease in the mean MOCART score from 6 months to 24 months (p=0·0312; figure 4B).

The glycosaminoglycan content of the repair tissue increased significantly from 6 months to 24 months, indicating compositional maturation towards hyaline cartilage. This change led to a reduced deviation from normal cartilage in the same joint, in which glycosaminoglycan content remained stable, as assessed by relative ΔR1 approaching the ideal level of 1 (significant decrease from mean 1·61 [SD 0·43] at 6 months to 1·38 [0·38] at 24 months; p=0·0073; figure 4C). The water and collagen content of the repair tissue also indicated a composition comparable with normal native cartilage (figure 4D). No association was noted between compositional characteristics (ie, glycosaminoglycan or water and collagen content) and morphological characteristics (ie, level of defect filling or MOCART score) of the repair tissue. Representative MRI scans for one patient (figure 4E) show that the extensive original cartilage defect in the trochlea was filled completely at 24 months. Quantitative T1 maps show comparable contrast enhancement between the repair tissue and the adjacent cartilage of the trochlea and patella.

New cartilage lesions observed by MRI in one patient (independent of the original lesion) required an arthroscopic assessment at 17 months after the initial operation. The defect was filled to the level of the surrounding native cartilage with a slight irregular surface (appendix p 2). Palpation with an arthroscopic hook revealed (subjectively to the surgeon) comparable tactile properties compared with the surrounding cartilage. A second-look biopsy at the site of engineered graft implantation was harvested; histological analysis indicated heterogeneous repair tissue without the typical architectural organisation of articular cartilage. However, in at least 50% of the tissue, cells were predominantly round, surrounded by lacunae in an extracellular matrix, which stained intensely for glycosaminoglycan and type II collagen, with only faint or absent staining for type I and type X collagens (appendix p 2). Although based on one occurrence, and at a stage of probable

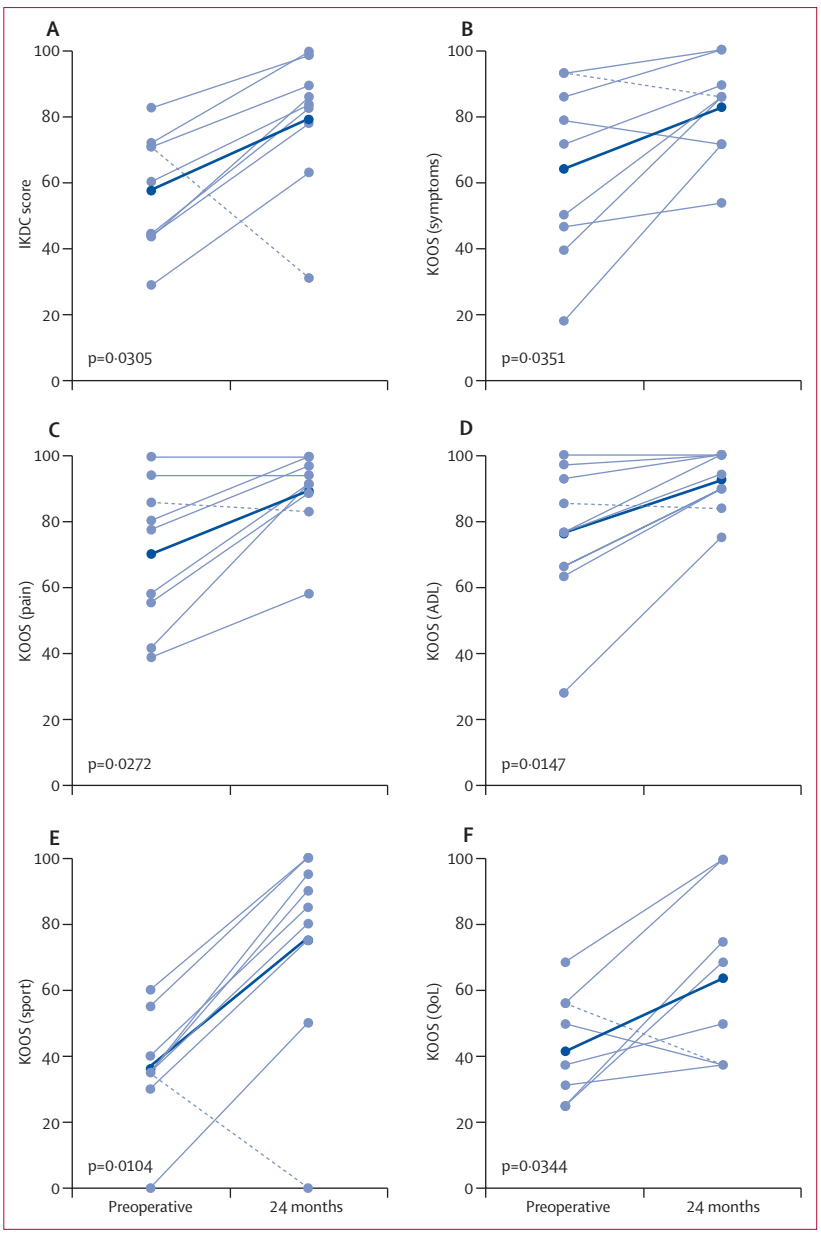
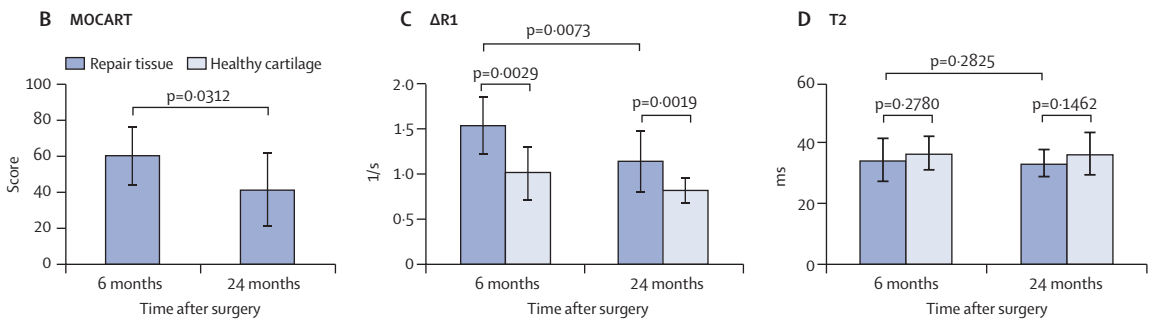


Figure 3: **Change in clinical scores from before surgery to 24 months after surgery**
Thick lines show mean values. Dashed lines show results from one patient who had to undergo a new surgical procedure for an independent cartilage lesion in the same knee. Significance was assessed by paired *t* tests (A, B, D, F) or Wilcoxon signed rank tests (C, E). IKDC=International Knee Documentation Committee. KOOS=Knee injury and Osteoarthritis Outcome Score (further classified for symptoms, pain, activities of daily living [ADL], function in sport, and quality of life [QoL]).

A

		Defect number										
		2	3	4a	4b	5a	5b	6	7	8	9	10
Defect filling	**6 months**	4	3	3	4	4	4	4	4	1	4	4
	24 months	4	4	4	3	3	1	4	1	1	5	3
Relative ΔR1	**6 months**	2·13	1·74	1·30	1·16	1·28	2·24	1·59	2·09	1·88	0·85	1·44
	24 months	1·33	0·93	1·03	1·86	1·67	1·69	1·12	1·48	1·38	1·24	1·50



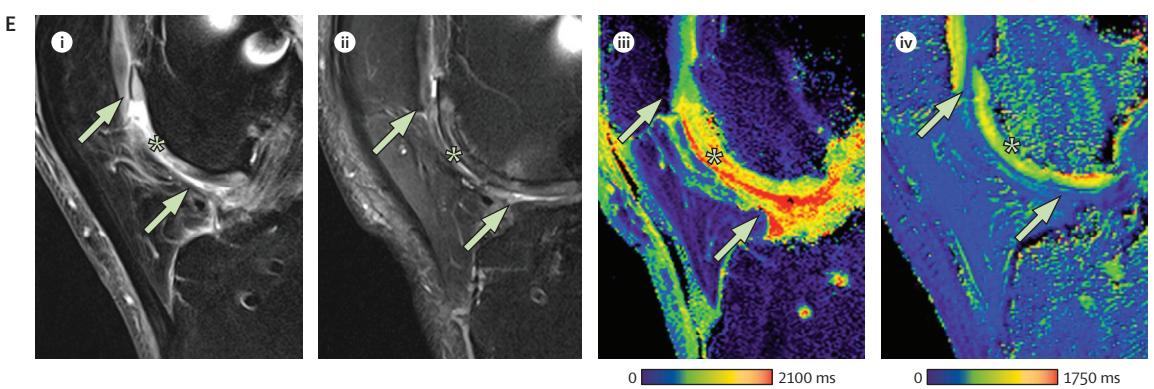


Figure 4: **Radiological assessments**
(A) Degree of defect filling: 0=graft lost (empty defect); 1=partial exposed subchondral bone; 2=incomplete with less than 50% filling; 3=incomplete with more than 50% filling; 4=complete filling (100%); 5=hypertrophic. Relative ΔR1 values calculated for every lesion, using delayed gadolinium-enhanced MRI of cartilage. (B) Magnetic Resonance Observation of Cartilage Repair Tissue (MOCART) scores, (C) ΔR1, and (D) T2 relaxation times are mean (error bars are SD), with significance assessed by paired *t* tests. (E) Proton density weighted images (i) before surgery and (ii) 24 months after surgery, and quantitative T1 maps (iii) before and (iv) after contrast administration in one patient at 24 months. Asterisks indicate the defect area, arrows indicate the border with adjacent cartilage. T1 maps display the quantitative T1 relaxation times in colour code, from blue (low) to green and yellow to red (high).

ongoing maturation, these findings argue for a hyaline nature of the cartilage tissue formed, at least in some areas of the repair site.

Discussion

We have shown that use of cartilage tissue engineered from autologous nasal chondrocytes for clinical repair of traumatic knee cartilage defects is feasible and safe. In our small cohort of patients for this phase 1 study, despite the variable degree of defect filling, self-assessment scores and MRI quantitative analyses established a satisfactory clinical outcome and a gradually improving quality of repair tissue over time.

Cellular therapies for treatment of traumatic cartilage defects are currently based on delivery of cells (predominantly articular chondrocytes[4] or mesenchymal stromal progenitors)[23] as a liquid suspension or through biodegradable matrices. In these grafts, even when a pre-culture time was introduced,[24] cells do not have a mature chondrogenic phenotype and are not embedded within hyaline-like extracellular matrix.[25] Our study, using highly chondrogenic cells (ie, nasal chondrocytes) and appropriate in-vitro processing,[7] is the first to test implantation of a mature engineered cartilage tissue for treatment of articular cartilage defects. The tissue-like properties of the graft, although associated with the need for mini-arthrotomy and incompatible with an injectable delivery mode, allowed practical handling and fixation with sutures during surgery, similar to alar lobule reconstruction.[9] The presence of abundant extracellular matrix that was produced by nasal chondrocytes during in-vitro maturation might have contributed to protect the

implanted cells from the harsh environmental conditions (including inflammatory and mechanical signals) at the site of an injured joint. Thus, consistent with data from animal models,[26,27] the extracellular matrix might have had a pivotal role in maintaining or improving over time the quality of the repair tissue.

Limitations of our study include the small number of patients enrolled, the fairly short observation time with no mechanical tests, and the absence of a control group, so a placebo effect with improvement of self-assessed clinical scores cannot be excluded. However, although the increase in clinical scores was in the range of previously reported outcomes using established cellular therapies,[28] the observed stability of T2 values represents a distinct improvement from the outcome of autologous articular chondrocyte implantation, for which a decrease from 6 months to 24 months was reported.[29] Moreover, the quantified deviation in glycosaminoglycan content between repair tissue and native cartilage (mean rΔR1 1·38) was better than that reported after microfracture (3·39) or matrix-induced autologous chondrocyte implantation (MACI; 2·18).[30] To define whether the observed clinical outcome can be attributed to use of nasal chondrocytes (*vs* articular chondrocytes) or the stage of tissue maturation, or both, a phase 2, multicentre, clinical study (NCT02673905) is underway. Such a trial will be important to identify graft parameters that should be set as release criteria to predict clinical potency, and further comparative studies will be needed subsequently to test possible superiority to conventional therapies.

In a preclinical in-vivo study,[31] grafting of tissues engineered from nasal chondrocytes in full-thickness articular cartilage defects resulted in preserving the typical structural organisation of the underlying subchondral bone, avoiding the sclerotic thickening associated with the onset and development of osteoarthritis. Longer follow-up of treated patients will enable us to assess whether engineered nasal cartilage implantation would also result in retardation or elimination of post-traumatic development of osteoarthritis in a clinical setting. The possibility to extend indications of engineered grafts to stages of early osteoarthritis,[32] to delay or eliminate the need for prosthetic implants, represents a major challenge for the developed method and a good opportunity for this area of research. Together with development and implementation of advanced manufacturing strategies to automate and control production processes under GMP compliance,[33] this perspective represents a crucial issue to show cost-effectiveness and gain acceptance by health insurance systems.

Contributors

MM coordinated all clinical tasks, follow-up, and data collection and analysis. AB, MM, and SM did the literature search and wrote the report. AW organised the quality management system for the graft production and clinical trial. SF, FW, and AMA validated the protocols for graft manufacturing, produced the tissues, and did histological, immunohistochemical, and biochemical analyses. DB, OB, and MK were responsible for the graft release and radiological assessments. GP did joint surgical operations. MH and DJS organised harvesting of biopsy specimens and blood collection. IM was the primary investigator of the study and lead author in writing the report. MJ was the primary clinical investigator and was responsible for all surgical operations. All authors have approved the final version of the report.

Declaration of interests

We declare no competing interests.

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