Engineered autologous cartilage tissue for nasal reconstruction after tumour resection: an observational first-in-human trial

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Summary

Background **Autologous native cartilage from the nasal septum, ear, or rib is the standard material for surgical reconstruction of the nasal alar lobule after two-layer excision of non-melanoma skin cancer. We assessed whether engineered autologous cartilage grafts allow safe and functional alar lobule restoration.**

Methods **In a first-in-human trial, we recruited five patients at the University Hospital Basel (Basel, Switzerland). To be eligible, patients had to be aged at least 18 years and have a two-layer defect (≥50% size of alar subunit) after excision of non-melanoma skin cancer on the alar lobule. Chondrocytes (isolated from a 6 mm cartilage biopsy sample from the nasal septum harvested under local anaesthesia during collection of tumour biopsy sample) were expanded, seeded, and cultured with autologous serum onto collagen type I and type III membranes in the course of 4 weeks. The resulting engineered cartilage grafts (25 mm×25 mm×2 mm) were shaped intra-operatively and implanted after tumour excision under paramedian forehead or nasolabial flaps, as in standard reconstruction with native cartilage. During flap refinement after 6 months, we took biopsy samples of repair tissues and histologically analysed them. The primary outcomes were safety and feasibility of the procedure, assessed 12 months after reconstruction. At least 1 year after implantation, when reconstruction is typically stabilised, we assessed patient satisfaction and functional outcomes (alar cutaneous sensibility, structural stability, and respiratory flow rate).**

Findings **Between Dec 13, 2010, and Feb 6, 2012, we enrolled two women and three men aged 76–88 years. All engineered grafts contained a mixed hyaline and fibrous cartilage matrix. 6 months after implantation, reconstructed tissues displayed fibromuscular fatty structures typical of the alar lobule. After 1 year, all patients were satisfied with the aesthetic and functional outcomes and no adverse events had been recorded. Cutaneous sensibility and structural stability of the reconstructed area were clinically satisfactory, with adequate respiratory function.**

Interpretation **Autologous nasal cartilage tissues can be engineered and clinically used for functional restoration of alar lobules. Engineered cartilage should now be assessed for other challenging facial reconstructions.**

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Introduction

Non-melanoma skin cancer—basal-cell carcinoma and squamous-cell carcinoma—is the most frequent malignant skin neoplasm in white individuals.[1] 2·2 million patients were treated for the disease in the USA in 2006, and the number of cases is increasing.[2] Most non-melanoma skin cancers arise in body areas exposed to sun, with the highest regional frequency (36%) on the nasal alar lobule.[3] The standard treatment is total local excision of the cancer. However, tumour removal could lead to a defect in the subcutaneous tissue and underlying fibromuscular fatty tissue—a two-layer defect—and loss of more than half the alar lobule. In these cases, the invasive procedure not only affects the structural integrity of the lobule, but also compromises its function. Moreover, changes in the aesthetical subunits of the nose can distress the patient.[4] Driscoll and Baker[5] stated that these factors "make aesthetic and functional reconstruction of the nasal alar defects one of the most challenging endeavors in facial reconstructive surgery".

After tumour excision, tissue loss requires a two-layer reconstructive approach, typically based on the use of a skin flap covering a structural support in the form of an allogeneic graft, a synthetic material, or an autologous cartilage graft. Use of allogeneic grafts can lead to immune rejection, disease transmission, and resorption.[6,7] Biocompatible synthetic materials are not associated with donor-site morbidity or immune rejection, but are associated with infection, extrusion, and, less commonly, foreign-body reaction, leading to suboptimum outcome.[7–9] Autologous cartilage grafts are deemed the best replacement for the stiff, fibromuscular fatty tissue that makes up the alar lobule, because they create contour, prevent collapse, and resist forces of contraction.[4–6,10–12] They are typically harvested from the auricular concha, rib, or nasal septum.[13] However, this technique presents the main disadvantages of an additional surgery and donor-site morbidity.

Engineered cartilage tissue might help to overcome the drawbacks associated with the use of native cartilage.

Many autologous chondrocytes can be generated from a small cartilage biopsy sample taken from the nasal septum, through expansion in the presence of specific growth factors and autologous serum.[12–14] Seeding and culture of expanded cells into appropriate matrices which provide structural cues together with biochemical factors guiding cellular differentiation generate cartilage grafts with clinically relevant sizes and mechanical properties that can mature further on subcutaneous implantation.[15–17] However, in spite of impressive images of cartilage structures growing ectopically in various animals,[18] the application of engineered cartilage tissues for the durable restoration of craniofacial structures has not yet been reported in the clinical setting.

On the basis of detailed preclinical experiments, we aimed to assess the safety and feasibility of autologous engineered cartilage as a replacement for native cartilage in the reconstruction of a two-layer defect of the nasal alar lobule. We also assessed aesthetic and functional outcomes at least 1 year after reconstruction, at a time when soft tissue structures have typically developed to a stable condition.

Methods

Study design and participants

In a first-in-human trial, we planned to recruit five patients with a two-layer defect after excision of non-melanoma skin cancer on the alar lobule at the University Hospital Basel (Basel, Switzerland). Panel 1 lists full inclusion and exclusion criteria. All participants provided written informed consent. For comparisons with a native reference material, we harvested alar lobule tissue from a female cadaver donor (aged 60 years) during autopsy, after approval by the local ethical committee and written informed consent was provided by relatives.

This study conforms with the Declaration of Helsinki, was approved by the ethical committee of Basel (EKBB 241/06) and by the Swiss Agency for Therapeutic Products (Swissmedic, TpP-I-2010-002, manufacturing authorisation number 32330). The clinical trial followed the European guidelines about advanced therapeutic medicinal products.

Panel 1: Inclusion and exclusion criteria

Inclusion criteria

- Defect ≥50% size of alar subunit
- Defect affecting skin and fibromuscular fatty tissue (two-layer defect)
- Age ≥18 years

Exclusion criteria

- Evidence of infection with HIV or hepatitis B or C
- Known allergies to porcine collagen, penicillin, or streptomycin
- Pregnancy or breastfeeding
- Evidence of syphilis
- Chronic treatment with steroids or immunomodulatory drugs

Procedures

To isolate chondrocytes, we harvested autologous septal cartilage when obtaining a biopsy sample from the suspicious skin lesion under local anaesthetic. The L-shaped mucosa incision was placed about 1 cm behind the anterior margin of the septum. A 6 mm cartilage biopsy sample was obtained after continuing the preparation in a subperichondrial plane, followed by closure of the mucosa incision with resorbable sutures. On the same day, we obtained 72 mL of venous blood to prepare autologous serum. We transported the cartilage biopsy sample and the blood to a clean room for graft manufacturing in line with good manufacturing practices, according to the defined standard operating procedures and in compliance with the established quality management system.

The cartilage biopsy sample was cut into pieces and incubated overnight in complete medium for sterility control. Complete medium consisted of Dulbecco's modified Eagle's medium (Gibco, Paisley, UK), containing 100 U/mL penicillin, 100 mg/mL streptomycin, and 29·2 mg/mL L-glutamine (Gibco, Grand Island, NY, USA) and supplemented with 5% autologous serum. We isolated chondrocytes with 0·15% type II collagenase (Worthington Biochemical Corporation, Lakewood, NJ, USA) for 22 h at 37°C. We expanded cells for 2 weeks in complete medium further supplemented with 5 ng/mL fibroblast growth factor 2 and 1 ng/mL transforming growth factor b1 (R and D Systems, Minneapolis, MN, USA), which has previously been shown to increase proliferation and post-expansion differentiation capacity.[19] We changed medium twice a week. We replated cells on the first 2 days and then 1 week after initial digestion by sequential treatment with 0·3% type II collagenase, and 0·05% trypsin and 0·53 mM EDTA (edetic acid; Gibco, Paisley, UK). Two operators manually counted cells according to a validated procedure and established cell viability using trypan blue 0·4% (Sigma Chemical, St Louis, MO, USA).

We seeded expanded cells on the porous side of Chondro-Gide, a CE-marked, licensed membrane consisting of porcine-origin collagen type I and type III (Geistlich Pharma, Wolhusen, Switzerland), at the previously defined density of 25 million cells per 25 mm×25 mm×2 mm membrane.[20] Because of the arrangement of the collagen fibres, the bilayer membrane has high tensile strength. It has been widely used for articular cartilage repair and supports chondrocyte differentiation. The porous layer of the membrane enables cell seeding, invasion, and matrix deposition, whereas the underlying compact and smooth layer prevents their dispersion. For each patient, we cultured cells in parallel in two membranes for 2 weeks using complete medium further supplemented with 10 µg/mL

insulin (Novo Nordisk, Bagsvaerd, Denmark) and 0·1 mM ascorbic acid 2-phosphate (Sigma Chemical, St Louis, MO, USA), to support matrix deposition and assembly. We changed media twice a week.

We undertook regular sterility controls throughout the manufacture. Three BacT/ALERT anaerobic and aerobic tests (bioMérieux, Durham, NC, USA) were done for each patient in the microbiology laboratory at University Hospital Basel: one after overnight biopsy incubation, one after 2 weeks of cell expansion, and one 3 days before implantation. Three VenorGeM PCR tests for mycoplasma detection (Minerva Biolabs, Berlin, Germany) were done at the Institute for Molecular Biology in Homburg, Germany, at the same times as for microbiology, except for the last one, which was done 1 week before implantation.

Quality control tests for release of the grafts included establishment of the absence of any contamination of cultured media, a 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 extracellular matrix. We estimated the proportion of viable cells by frozen section analysis (haematoxylin and eosin staining) of a 4-mm-wide stripe resected from the margin of the engineered grafts on the day of the implantation. On the basis of these criteria, which of the two grafts should be implanted by surgeons was established.

We took at least four samples from different regions of the non-implanted, spare construct for histological and biochemical assessments. We fixed samples for histology in 4% formalin, embedded them in paraffin, and took cross-sections (7 µm thick). We stained sections for sulphated glycosaminoglycans with Safranin O or processed them for immunohistochemistry with antibodies against collagen type II (mouse anti-human, clone II-4CII, MP Biomedicals, Santa Ana, CA, USA), type I (mouse anti-human I, clone I-8H5, MP Biomedicals, Santa Ana, CA, USA), or type X (mouse anti-human, clone ab49945, Abcam, Cambridge, UK). Samples for biochemical analysis were weighed and digested with proteinase K as previously described.[21] We measured glycosaminoglycan content with a spectrophotometer after reaction with dimethylmethylene blue, with chondroitin sulphate as a standard. We normalised the glycosaminoglycan content to the DNA amount, which was measured with the CyQUANT cell proliferation assay kit (Molecular Probes, Eugene, OR, USA), with calf thymus DNA as a standard. We also normalised the amount of glycosaminoglycans to the tissue wet weight.

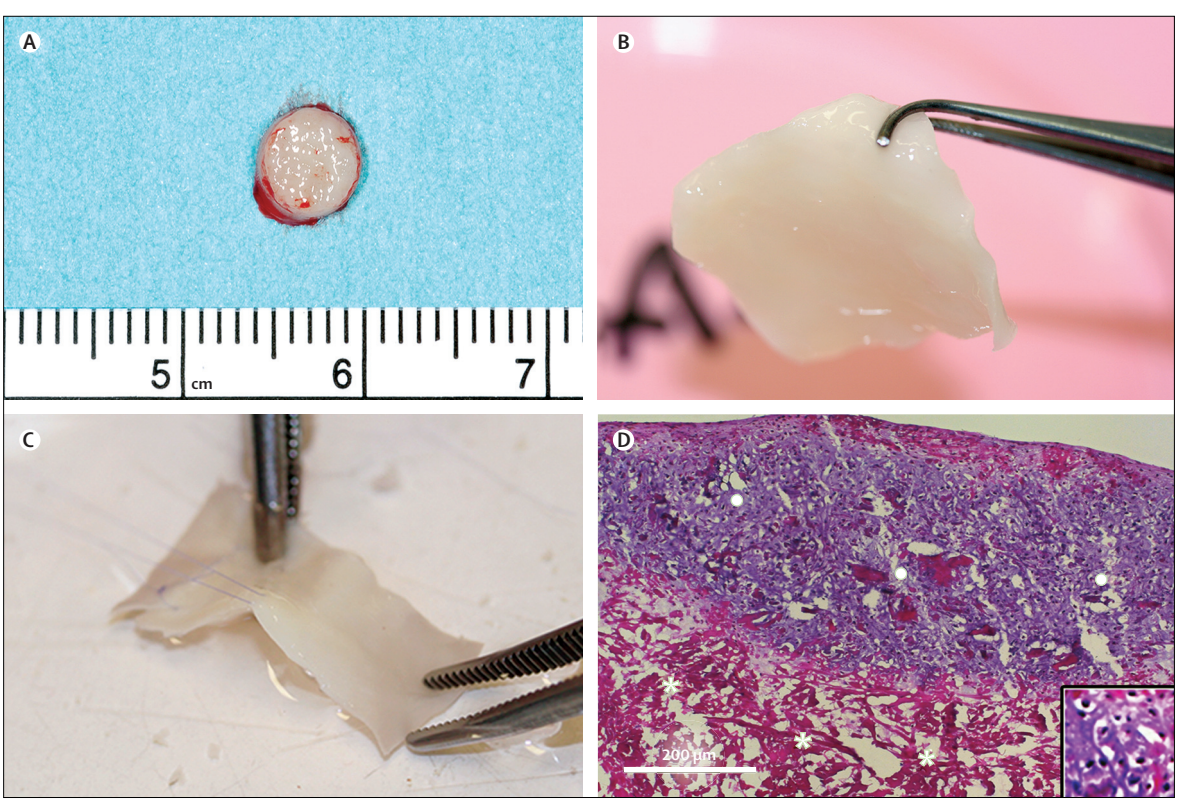

Figure 1: **From the nasal biopsy to the engineered cartilage graft**
(A) Macroscopic view of the biopsy sample (6 mm diameter) of nasal septum cartilage. (B) White and glossy appearance of an engineered cartilage graft. (C) Qualitative handling and suturing tests. (D) Haematoxylin and eosin staining of frozen sections to fulfil the release criteria for graft implantation. Asterisks indicate the compact layer of the membrane, which is predominantly cell free; circles indicate the porous layer of the membrane, including cells and the deposited extracellular matrix (see inset for image at four-times higher magnification).

After basal-cell carcinoma or squamous-cell carcinoma was histologically verified, tumour excision was done under local anaesthesia as an outpatient procedure 1 week before reconstruction. We planned the reconstruction in two stages, with the goal of a normal facial contour, using templates for design.[4] The surgical and postoperative protocols were identical to the standard of care, with the exception that the engineered cartilage was implanted instead of a native cartilage graft to prevent collapse of the structure and resist forces of contraction. The engineered cartilage was shaped according to the defect, implanted with the cell-seeded side towards the mucosa with the assumption that this placement would guarantee more efficient cell nutrition, and secured on the edges with absorbable sutures (Biosyn 5/0, Covidien, Dublin, Ireland). We reconstructed the outer layer as necessary, with either a randomised nasolabial flap or a paramedian forehead flap. For paramedian forehead flaps, the flap was autonomised 2 weeks later in an additional procedure (total of three stages), with division of the pedicle.

6 months after defect reconstruction, we did a flap refinement as the last stage, with subcutaneous sculpturing that allowed a biopsy sample of the implanted graft to be obtained simultaneously. We harvested the second-look biopsy sample (3 mm×3 mm; down to the mucosal lining) with a scalpel from the most cephalic area of the implanted construct to avoid irregularities in the alar rim and to prevent possible weakening of the alar lobule stability. We fixed these biopsy samples and the native alar tissue, embedded them in paraffin, took cross-sections, and stained them with haematoxylin and eosin or Safranin O.

Outcomes

The primary outcomes of the trial were safety and feasibility of the procedure, assessed 12 months after reconstruction. We established safety by reports of adverse events and their further classification into serious adverse events or serious adverse reactions on the basis of guidance from the European Medicines Agency.[22] All adverse events, either local (eg, infection or haematoma) or systemic (eg, fever or allergic reaction), would be handled according to the Guidelines for Good Clinical Practice from the International Conference on Harmonisation and the Verordnung über klinische Versuche mit Heilmitteln. We defined feasibility on the basis of three factors: graft manufacturing with patient-derived autologous cells and serum; surgical manipulation of the graft with a typical two-layer alar lobule reconstruction; and preservation of structural stability, which is necessary for a clinically satisfactory breathing function and aesthetic outcome.

We had two secondary outcomes: patient satisfaction after 12 months measured by self-assessment with the visual analogue scale in terms of breathing, aesthetic appearance, and pain after 12 months; and alar cutaneous sensibility, structural stability, and respiratory flow rate assessed 18–30 months after reconstruction. We assessed

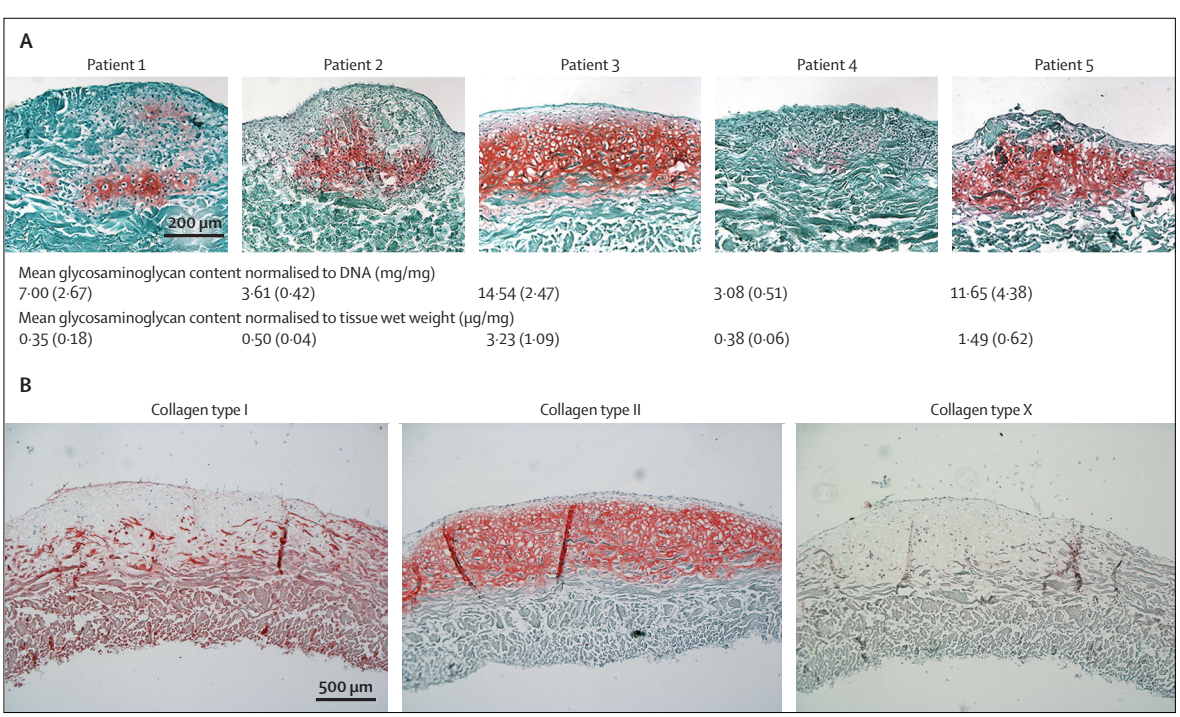

Figure 2: **Histological and biochemical characterisation of engineered grafts on implantation**
(A) Safranin O staining of engineered grafts obtained for each patient at time of implantation; data in parentheses are SD. (B) Immunohistochemical staining for collagen type I, II, or X in the graft engineered with cells from patient 3.

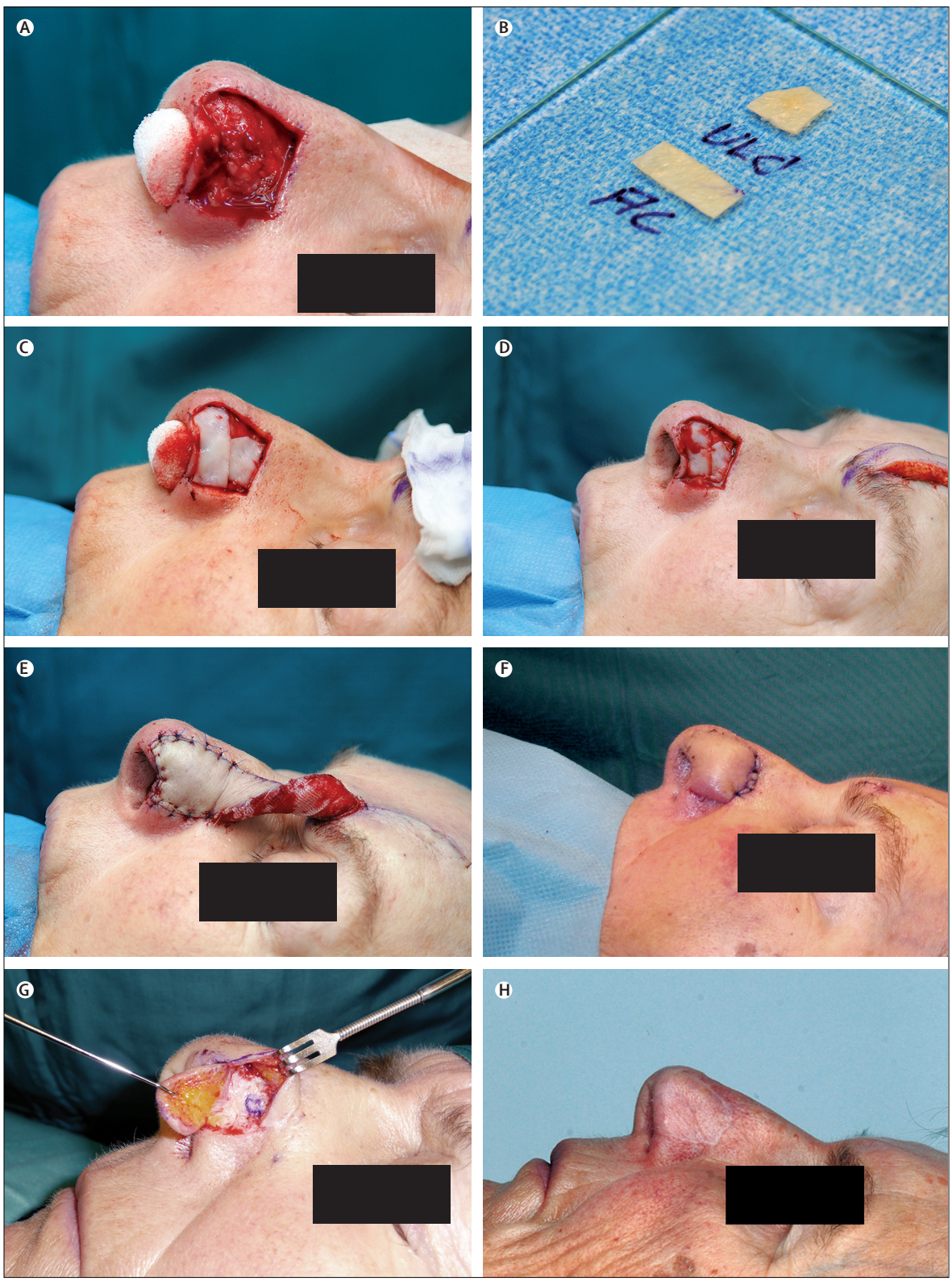

Figure 3: **Surgical procedure in one patient**
(A) Two-layer defect after wide local excision of the skin cancer on the alar lobule. (B) Tissue engineered cartilage cut to the right shape and ready for implantation; this patient needed cartilage support to achieve stability in the alar lobule (labelled AC) and at the upper lateral site (labelled ULC). (C,D) Tissue engineered cartilage was inserted to replace the structural support and secured by absorbable sutures. (E) Reconstruction of the outer layer with a paramedian forehead flap. (F) Division of the flap pedicle 2 weeks after reconstruction. (G) Intra-operative appearance of the implanted engineered tissue during refinements 6 months after reconstruction. (H) Follow-up 1 year after reconstruction.

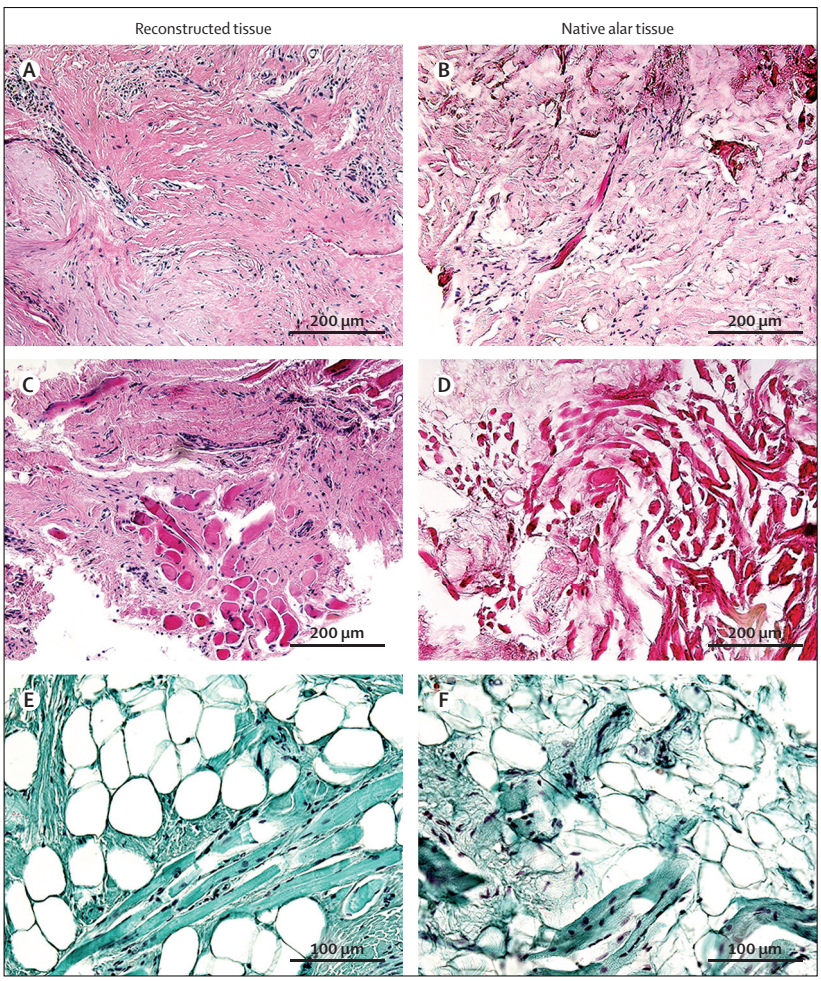

Figure 4: **Histological analysis of reconstructed and native alar tissue**
Biopsy samples from the reconstructed tissue 6 months after graft implantation (A, C, E) and from native alar tissue (B, D, F) had similar structures. (A, B) Fibrous connective tissue; haematoxylin and eosin staining. (C, D) Muscle fibres; haematoxylin and eosin staining. (E, F) Fat cells; Safranin O staining.

cutaneous sensibility, which is directly dependent on the flap, to exclude a possible negative effect of the graft on the flap.

We assessed cutaneous sensibility and stability of the nostrils on the reconstructed region at the centre of the alar lobule with Semmes-Weinstein monofilaments (Homecraft Rolyan, Sutton in Ashfield, UK)[23] of increasing diameters, ranging from 1·65 mm to 6·65 mm, corresponding with the application of weights from 4·5 mg to 447 g. For sensibility tests, patients closed their eyes and were instructed to respond when they felt the applied monofilament. For stability tests, monofilaments were held perpendicular to a silicon plate (Epiform flex, Institut Schilling, Neubrunn, Germany; 1 cm², 2 mm thick) glued onto the nasal alar lobule and pushed until collapse of the alar lobule. For functional assessments, we recorded the smallest monofilament size that the patient could detect (at least two of three stimuli) or that resulted in total collapse of the lobule.

After exclusion of a septal perforation, a complete obstruction of a nasal cavity, or ventilation-impairing disorders by anterior rhinoscopy, we assessed the respiratory function of the nose by active anterior rhinomanometry. The dynamic test measures the pressure difference between the posterior and the anterior parts of the nose (Δp) and the nasal airflow (V) at a specific pressure. We took measurements during active spontaneous breathing before and after application of Tinaphin (naphazoline and tetracaine; Spital Pharmacy, University Hospital Basel) in each nostril as topical decongestant and anaesthetic. Nasal airflow resistance was expressed as R=Δp/V, assessed at 150 Pa on the inspiratory part of the rhinomanometry.[24] A moderate obstruction corresponds to a resistance of 0·5–0·8 cm³/s and a severe obstruction to a resistance of higher than 0·8 cm³/s.

This study is registered with ClinicalTrials.gov, number NCT01242618.

Role of the funding source

The funders of the study had no role in study design, data collection, data analysis, data interpretation, or writing of the report. The corresponding author had full access to all the data in the study and had final responsibility for the decision to submit for publication.

Results

Between Dec 13, 2010, and Feb 6, 2012, we enrolled two women and three men aged 76–88 years who presented with either basal-cell carcinoma (n=4) or squamous-cell carcinoma (n=1). Skin lesions were up to 18 mm×15 mm in size.

For the supplementation of culture medium, a minimum volume of 25 mL of autologous serum was necessary and systematically obtained (mean serum volume 37·4 mL [SD 6·6]). Expansion of the chondrocytes isolated from the nasal septum cartilage biopsy samples (0·28 cm²; figure 1A) according to the defined protocols led to a mean of 84·4 million cells per patient (SD 11·9 million; mean viability 99·3% [SD 0·5]). Therefore, despite the reduced cellularity in nasal septum cartilage in elderly patients,[25] cell numbers were always more than sufficient for the production of two engineered cartilage constructs per patient (12·5 cm² in total).

In-process controls based on sterility tests identified no microbiological or mycoplasma contamination for any manufacturing batch. The produced cartilage grafts maintained the initial scaffold size and shapes, had a white and glossy appearance, and were sufficiently stable to be manipulated with forceps and sutured without any rupture (figure 1). The deposition of extracellular matrix containing at least 70% viable cells confined within the porous layer of the Chondro-Gide membrane was verified for each graft on the basis of frozen section analysis within 20 min after tissue sampling (figure 1D). Therefore, each manufactured graft fulfilled the defined release criteria for implantation.

In histological assessments of the non-implanted constructs, all tissues stained positively for glycosaminoglycans, although to a variable intensity and with little spatial uniformity (figure 2A). The cartilaginous nature of the deposited matrix and the interpatient variability were confirmed by the biochemical quantification of glycosaminoglycans (figure 2A). Further immunohistochemical analyses indicated that the extracellular matrix produced had features of a mixed hyaline and fibrous cartilage. We detected collagen type II in areas positively stained for glycosaminoglycans, whereas collagen type I staining was positive in regions containing elongated cells, mostly at the construct periphery (figure 2B). We did not detect collagen type X in the grafts (figure 2B), indicating that the cartilaginous tissue was not developing towards a hypertrophic stage. We did not detect p53, a transcription factor usually expressed in transformed cells, or the antigen Ki-67, a marker of cell proliferation, in engineered grafts (data not shown).

Total excision of a non-melanoma skin cancer led to a two-layer defect in all five patients. The defects included more than 50% of the area of the alar lobule, and also involved the nostril rim. The two-layer defects measured 1·7–2·3 cm (anterior to posterior) and 1·2–2·2 cm (cephalical to caudal). We tailored the released cartilage graft according to defect size and contour, which meant the graft was cut into one or two parts with a mean total surface area of 3·8 cm². We implanted the grafts in the clean, well perfused pocket, which extended the size of the original defect towards the nasal tip and the alar base for improved anchoring, and used resorbable sutures for stitching (figure 3A–D). Two patients needed a paramedian forehead flap (figure 3E) and three patients needed a nasolabial flap to reconstruct the outer layer. Pedicle division of the paramedian forehead flap was done 2 weeks after reconstruction in an additional stage (figure 3F). To improve aesthetic outcome, we undertook flap refinement with subcutaneous sculpturing in all patients 6 months after reconstruction (figure 3G). After skin incision via the old scars, we elevated the flap and obtained a biopsy sample from the implanted graft, which could be macroscopically identified by a white and glossy appearance and by a dense texture on palpation. We excised some subcutaneous tissue from the flap to thin it out and adjust it to the shape of the contralateral healthy lobule.

Histological analysis of these biopsy samples and native alar lobule tissue indicated the formation of analogous structures consisting of fibrous connective tissue (figure 4A–B), muscle fibres (figure 4C–D), and fat cells (figure 4E–F), and the absence of cartilaginous matrix. Therefore, the graft remodelled into a thick fibromuscular fatty tissue on implantation, similar to that in the region where it had been implanted.[10,26–28]

After 12 months, no local or systemic adverse events had been recorded. The reconstructed alar lobules were

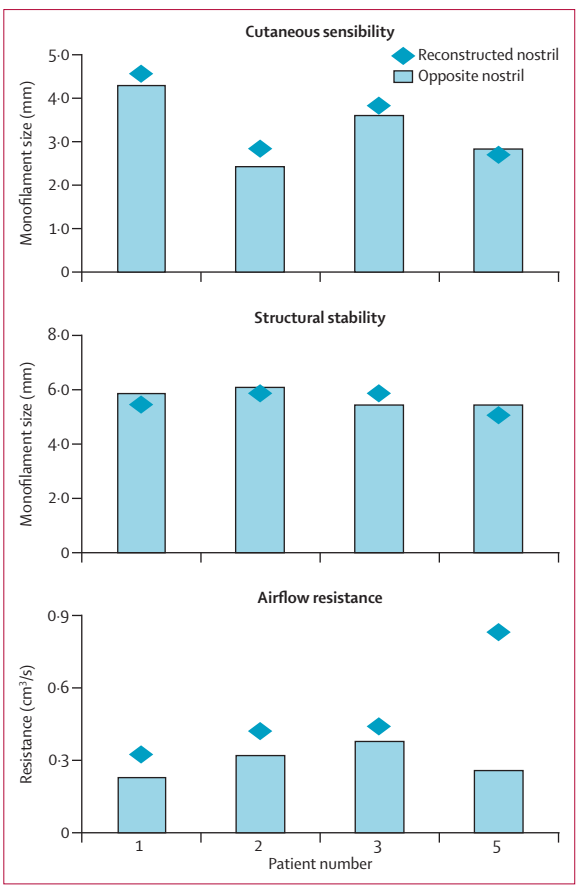

Figure 5: **Functional assessments after 18–30 months**
Data available for four of the five patients.

stable; in each case, they allowed a clinically satisfactory breathing function and aesthetic outcome. Patients did not notice changes in breathing capacity from the preoperative condition and did not have air restriction while doing daily activities or in forced inspiration. All patients were satisfied with the aesthetic appearance on the basis of restoration of shape and extent of scarring, and did not report pain (score of 0 on visual analogue scale for all patients).

We could not quantify cutaneous sensibility, structural stability, and airflow resistance in patient 4 because of ethical reasons: the patient had a poor general health status that was not related to the surgical treatment. In the other patients, the quantified cutaneous sensibility and structural stability of the reconstructed nostrils were clinically satisfactory and not significantly different from those of the opposite nostrils after at least 18 months (figure 5). Quantitative measurements of airflow by rhinomanometry in the reconstructed nostrils were clinically satisfactory, and resistance was only slightly higher than in the opposite nostrils (figure 5). In all cases, patients had a mild obstruction, with the exception of patient 5, who had a severe obstruction in the reconstructed nostril, which was due to a severe septal

deviation (present before the reconstruction), with subluxation of the lower margin into the ostium of the reconstructed side.

Discussion

Our findings show that use of autologous engineered cartilage tissues in the clinical reconstruction of the nasal alar lobule after resection of a non-melanoma skin cancer is safe and feasible. Self-assessment and quantitative tests of nasal function established that the clinical outcome was fully satisfactory in our small cohort (panel 2).

For reconstructive purposes, the fibromuscular fatty alar tissue is typically replaced by native cartilage, because of its capacity to resist contraction, improve contouring, increase volume, and stabilise the external nasal valve.[31] To clinically assess engineered autologous cartilage tissues as an alternative, protocols for production of structurally stable cartilaginous grafts first needed to be developed and validated. Notably, chondrocyte-based products in clinical use for articular cartilage repair consist of cell suspensions or of cells delivered by scaffold, but are not formed by frank cartilaginous extracellular matrix.[32] To ensure the quality of manufactured cartilage tissues has acceptable reproducibility, groups have previously preclinically defined media supplements to use during cell expansion for the preservation of the cell chondrogenic capacity,[19] a suitable density of cell seeding within porous three-dimensional scaffolds,[20] the effectiveness of autologous serum as a replacement of animal origin factors,[14] and the duration of culture to achieve sufficient mechanical properties and postimplantation stability.[15] These protocols were embedded within a quality management system compatible with the present Pharmaceutical Inspection Co-operation Scheme.

Therefore, one important outcome of our study is the proof of feasibility in the unprecedented engineering of human autologous nasal cartilage tissues suitable for a clinical test. The size of the generated cartilage (12·5 cm² for the two constructs) was more than 40-times larger than the original biopsy (0·28 cm²). The quality of the engineered tissues indicated some interdonor variability, as was expected on the basis of previous analyses.[14] The deposition of cartilaginous matrix was fairly inhomogeneous in the grafts, because of the inherent limitations of static cell seeding and culture within porous scaffolds. Tissue uniformity even with scaffolds thicker than 2 mm could be improved by the introduction of dynamic culture systems,[33] such as perfusion-based bioreactors.[34]

The implantation of engineered cartilage was sufficient to provide adequate and durable stability of the nostril. Although reference values for the quantitative tests after use of native cartilage are not available, our clinical results were similar to those after reconstruction with native cartilage. A comparison with the scaffold alone would not be ethically acceptable, because a soft material like Chondro-Gide is not deemed appropriate for reconstruction. Indeed, our findings indicate that the stiffness of the engineered grafts had reached a suitable threshold that is necessary to prime a favourable clinical outcome,[35] thanks to the deposited extracellular matrix.[15] At the same time, the known suboptimum results derived from adequately stiff but synthetic matrices[8] suggest that the cultured chondrocytes or deposited matrix, or both, had an active role in the formation of a tissue similar to that of the alar lobule. The implanted fibrocartilaginous grafts were remodelled into fibromuscular fatty structures similar to the tissue at the site of implantation. The absence of cartilage after 6 months could be because of the absence of environmental factors that are necessary for maintenance or further development of the cartilaginous phenotype, or both. In goats, engineered nasal cartilage grafts implanted in articular surface defects in the knee remain cartilaginous (Pelttari K, University Hospital Basel, personal communication). Therefore, the chemicophysical conditions at the site of implantation could regulate the fate of the engineered cartilage. Additionally, no data are available for the phenotypic stability of native cartilage grafts when implanted in the alar lobule environment as far as we are

Panel 2: Research in context

Systematic review

We systematically searched Medline for reports published in any language between Jan 1, 1996, and Dec 31, 2013, with the terms "tissue engineering" and "cartilage". The number of relevant reports has risen greatly in the past 10 years, up to 656 in 2013. When we restricted our search to clinical studies, we identified a few reports that were almost exclusively related to tracheal regeneration or joint resurfacing. We identified no report describing the clinical implantation of engineered cartilage tissue to reconstruct craniofacial structures. In plastic and reconstructive surgery, chondrocytes have been used only as cell suspensions—eg, for augmentation rhinoplasty.[29] From the original report of engineered cartilage in the shape of a human ear,[18] progress has been confined to relevant but still preclinical steps forward (eg, the neovascularisation of engineered auricle grafts with a skin flap in rabbits),[30] which remain rather distant from clinical implementation.

Interpretation

We have shown that engineered tissues containing cartilage extracellular matrix can be created from patients' own nasal chondrocytes and can be safely used to reconstruct craniofacial structures. The aesthetic and functional outcomes were fully satisfactory in five patients, and extended studies with larger cohorts are now needed. Facial reconstructive surgery frequently requires the use of the structural support offered by native cartilage grafts. Therefore, the reported proof-of-principle replacement of native with engineered cartilage tissues opens unprecedented paths for research.

aware. Further fundamental studies are necessary to investigate the fate of the implanted cells, their mechanism of action, and the specific properties to be reached by the engineered cartilage, which should inform more stringent release criteria.

In conclusion, we have reported for the first time that engineered cartilage tissue can safely replace native cartilage for the reconstruction of the alar lobule, leading to restoration of function, aesthetic satisfaction, and no donor-site morbidity. Our study opens the way to a controlled trial in which the long-term outcome of the procedure is prospectively compared with that of the gold-standard surgery and to the clinical assessment of engineered cartilage in other challenging facial reconstructions, such as those of heminose or complete nose, eyelid, or ear.[36] One important question to be addressed in future studies is the cost-effectiveness of a cell-based treatment when compared with the harvest of autologous native tissues. Engineering of autologous cartilage tissues is demanding and has high costs, but it could be justified by large benefits (eg, no risk of complications at an additional surgery site and increased patient satisfaction because of reduced morbidity). The analysis of economic sustainability should assess the possible introduction of automated and closed bioreactor systems for streamlined and controlled manufacturing[37,38] to minimise operator handling and potentially reduce costs of personnel and clean room space.

Contributors

IF and MDH undertook all clinical tasks, including harvesting of biopsy, blood collection, graft surgery, clinical follow-up, and functional tests. IF and SM did the literature search. AB, SF, FW and AM validated the protocols for graft manufacturing, produced the tissues, and did histological, immunohistochemical and biochemical analyses. SM, AW, and MJ organised the quality management system for the clinical trial. JF contributed to study design and to the definition of the graft production protocols. GJ interpreted the histological results for the graft release. MH contributed to the study design and organised the required infrastructures. DJS supervised and was responsible for all surgical operations. IF, SM, and IM wrote the report. IM coordinated study design and implementation, and was responsible for the release of the grafts.

Declaration of interests

We declare that we have no competing interests.

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