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Cartilage regeneration using decellularized cartilage matrix: Long-term comparison of subcutaneous and intranasal placement in a rabbit model

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ABSTRACT

Autologous cartilage as donor tissue for various surgical reconstructions such as nasal septum regeneration is limited and associated with donor site morbidity. Our goal was to evaluate a new resorbable chondroconductive biomaterial made of decellularized porcine nasal septum cartilage compared with autologous native auricular cartilage as the gold standard. In order to examine the material and determine its long-term outcome further, we used subcutaneous implantation and septal implantation in an orthotopic rabbit model. In addition to non-seeded decellularized xenogenic cartilage, chondrocyte-seeded decellularized xenogenic cartilage was implanted as a septal replacement. After a three- or six-month period, the formation of newly synthesized cartilage extracellular matrix was evaluated immunohistochemically, whereas septal integrity and biocompatibility were evaluated histologically. The formation of the implanted neoseptum and form stability was analyzed by using 7-Tesla Magnetic Resonance Imaging.

Good biocompatibility with no excessive rejection was demonstrated in all groups. Long-term stable and reliable septal reconstruction could be achieved in the study groups with or without cell seeding with autologous auricular chondrocytes. Autologous cell seeding was advantageous only with regard to septal perforations. Thus, cell seeding provides a benefit regarding long-term stability. However, because of slightly better biocompatibility, less pronounced septum deviation and the markedly lower effort involved, the non-seeded scaffold is favoured for possible clinical application.

1. Introduction

Tissue engineering is a promising method for the repair or replacement of human tissue that is injured or damaged as a result of disease or trauma (von Bomhard et al., 2013; Morouco et al., 2016). Autologous cartilage from the rib, the nasal septum or the auricle is commonly used for plastic reconstructive surgery of the nose and auricle and for reconstruction of the larynx and trachea and at orthopaedic sites (Rotter et al., 2005). As the availability of appropriate autologous cartilage donor tissues is limited and associated with donor site morbidity (Langer and Vacanti, 1993; Rotter et al., 2007), worldwide interest in cartilage tissue replacement has become a main target in tissue engineering research (Rotter et al., 2007; Haisch et al., 2005; Liu et al., 2017). For the regeneration of larger cartilage defects, it is of the utmost importance that the scaffold material is individually shapeable and adaptable to the defect and that it enhances cellular function comparable with native tissue. The structural architecture of the scaffold should ideally mimic the native tissue. Moreover, the scaffold should support and facilitate the infiltration, attachment,

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proliferation and differentiation of the desired individual cell type (Benders et al., 2013). We have created a resorbable chondroconductive biomatrix made of decellularized cartilage (Breiter et al., 2010) that fulfills these requirements. Recently, we have also developed a suitable orthotopic animal model for nasal septum repair (Bermueller et al., 2013). To evaluate the invented scaffold in a large defect, we scaled our animal model. In order to design the study to be as clinically relevant as possible, we decided to adopt an autologous animal model and transplanted the cultivated cartilage tissue within a closed system. The biomaterial made of decellularized porcine nasal septum cartilage was implanted seeded with autologous auricular chondrocytes as well as non-seeded. Our goal was to evaluate the new biomaterial for nasal septum regeneration in the area of their later use – merely covered with a thin layer of mucous-membrane – compared with native cartilage as actual gold standard and with a subcutaneous implantation. We had the intention to achieve a clinically stable reconstruction – not necessarily by repopulation of the material as it could also serve as guidance substance and temporal cartilage replacement material.

2. Materials and methods

All reagents, cell culture media and supplements were purchased from Sigma Aldrich (Munich, Germany), Invitrogen (Darmstadt, Germany) and Biochrom (Berlin, Germany), unless indicated otherwise.

2.1. Xenogenic cartilage biomaterial

Processed and sterile porcine nasal septum cartilage (pNSC) was prepared at the Institute of Bioprocess Engineering of University of Erlangen, Germany as described elsewhere in detail (Schwarz et al., 2015). The harvested cartilage was decellularized and sterilized, lipids and proteins were removed and pathogenic agents such as prion proteins (PrP^{sc}) or HI virus were inactivated as described elsewhere in detail (Schwarz et al., 2012b). The amount of glucosaminoglycans (GAGs) was reduced with a patented wet chemical decellularization process (DP) by using sodium hydroxide (NaOH) and hydrogen peroxide (H_2O_2) (Breiter et al., 2010) to increase porosity and to enable the migration of cells into the scaffolds. The vitality of cells cultured in matrix extracts was shown to be above 90% as described elsewhere in detail (Schwarz et al., 2012b). To achieve homogenous reproducible scaffolds for septal replacement, processed sterile cartilage samples were frozen at −24 °C and cut, by using a cryotome (CM1950, Leica Biosystems), to a height of 350 μm appropriate to the thickness of native rabbit nasal septum cartilage. Scaffolds for subcutaneous implantation were cut to a height of 1 mm. Cryosectioning was performed after particularly cleaning of the cryotome with 80% ethanol and touched only with sterile gloves and instruments. The blade was sterilized prior to use. Following DP and sectioning, scaffolds were stored deep-frozen (−24 °C) in physiological sodium chloride solution until further use.

2.2. Harvesting of cartilage, cell culture and seeding of biomaterials

In a first operation, an auricular cartilage biopsy, 5 × 30 mm in size, from the right auricle of each rabbit in study groups 1 and 2 (see below) was harvested in order to obtain autologous chondrocytes. Cartilage was rinsed in expansion medium, namely Dulbecco's modified Eagle medium Ham's F-12 (DMEM/Hams-F12) supplemented with 0.5% gentamicin. Cartilage was minced into 0.5 × 0.5 mm pieces under sterile conditions and was transferred to digestion medium (DMEM/Hams-F12, containing 0.3% collagenase type II (Worthington)) and incubated for 18 h at 37 °C in a shaking water bath. The supernatant was filtered (BD-Falcon, 100 μm) and the remaining cell solution centrifuged. The generated cell pellet was resuspended in expansion medium. The total cell number and vitality were determined. Cells were seeded for amplification at a density of 0.5×10^4 cells/cm^2 and cultured at 37 °C and 5% carbon dioxide (CO_2). Following the attachment of the cells, culture medium was changed three times per week. Cells were used for seeding in passage 1.

A cell culture differentiation medium free of TGF-β was developed to obtain a medium with defined components (DMEM/Hams-F12 supplemented with 50 μg/ml ascorbic acid, 2.5 μg/ml insulin human, 46 μg/ml proline, 10% FBS and 0.5% gentamicin (PAA)) (Schwarz et al., 2012b). Commercially available media are very expensive and their composition is unfortunately largely unknown because of the secrecy of the manufacturers, this makes the assessment of results and the comparison with other media very difficult. Our TGF-β-free cell differentiation medium was judged to be very suitable, by means of Hoechst 33258 and histologically, and was therefore used for the experimental rabbit study.

Prior to seeding, decellularized xenogenic cartilage was sterilized in 80% ethanol for up to 2h at room temperature in order to exclude any contamination caused by shipping and thawing. To adjust pH value and rehydrate the scaffold matrix, scaffolds were incubated in the TGF-β-free three-dimensional (3D)-culture medium for 24h at 37 °C and 5% CO_2. Medium was removed and the scaffolds were seeded with 1×10^6 cells/cm^2 in 500 μL 3D-culture medium in a specially produced seeding device. The device was exactly fitted to the form and size of the scaffold and composed of glass, as glass is a bio-inert and easily sterilisable material. Fig. 1 shows the seeding device. Thus, the cells could be added at high density to the scaffold. After adding the cell suspension, the device was gently shaken once in order to distribute the cells homogeneously over the scaffold surface. Because of the precise position of

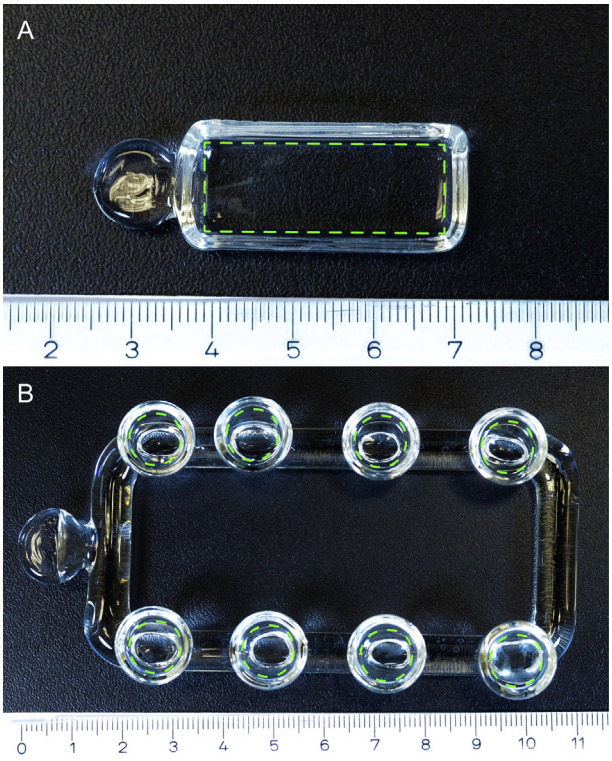

Fig. 1. Seeding devices A: for septal implants (1 cm × 3 cm × 350 μm); B: for subcutaneous discs (1 cm^2 × 1 mm). Green dashed lines show the position of pNSC in the seeding devices.

the scaffold in the glass vessel and because of surface tension, the cell suspension moistened the entire scaffold surface and nearly all cells became adherent. Another 500 μL 3D-culture medium was added after 1h to the scaffold. At 2h, the scaffold with adhered cells was transferred into a 6-well plate containing 3D-culture medium to remove all non-adhered cells. Medium was changed three times per week. The seeded scaffolds were pre-cultivated in the incubator for 14 days prior to implantation. Not the whole pre-incubation was done in the seeding device. Due to the small size of the device, the maximum amount of medium was limited, and the medium would have been used up too fast. To prevent a shortage of nutrition the scaffold was transferred into the 6-well plate after cells were adherent to the surface. Before implantation, the biomaterial constructs were rinsed by using an isotonic sodium chloride solution (B. Braun, Melsungen, Germany) in order to remove medium residues. Homogeneous cartilage constructs could thus be generated and nearly all amplified cells were applied to the scaffold at high density.

2.3. Animal model

The trials were conducted on female *Chinchilla Bastard rabbits* of 3 ± 0.5 kg in weight (Charles River Laboratories, Sulzfeld, Germany). This study was carried out in strict accordance with European guidelines for the Care and Use of Laboratory Animals. The protocol was approved by the Committee on the Ethics of Animal Experiments of the Regional Ethical Board in Tuebingen, (Permit Number: Reg. Ba-Wü. AZ - 1092/2012). All surgery was performed by means of an infusion of 40 mg/kg ketamine and 4 mg/kg xylazine i.v. and all efforts were made to minimize suffering. All operations were carried out under sterile conditions. Two implantation sites were evaluated for the cultured biomaterial in each animal: septal placement and four subcutaneous discs on the back. Two study groups and two control groups were performed with six animals in each group (three animals for each time point):

Study group 1: chondrocyte seeded decellularized xenogenic cartilage (approx. 1 cm × 3 cm × 350 μm) as septal replacement and four non-seeded decellularized xenogenic cartilage discs ($1\ cm^2 \times 1$ mm) subcutaneous on the back.

Study group 2: non-seeded decellularized xenogenic cartilage (approx. 1 cm × 3 cm × 350 μm) as septal replacement and four seeded decellularized xenogenic cartilage discs ($1\ cm^2 \times 1$ mm) subcutaneous on the back.

Control group 1: autologous native auricular cartilage (approx. 1 cm × 3 cm × 350 μm) as septal replacement and no implants on the back.

Control group 2: removal of the septum with no septal replacement and four autologous native ear cartilage discs ($1\ cm^2 \times 1$ mm) subcutaneous on the back.

The anaesthetized, spontaneously breathing, rabbits were arranged ventrally on their abdomen. To prevent a collapse of the nasal airway, the animals were nasally intubated by using a 14G indwelling venous cannula (Vasofix® Braunüle®, B.Braun Melsungen AG). To protect the cornea, ointment was administered in both eyes (Bepanthen®, 5% Dexapanthenol).

Septal implantation. After the fur had been shaved on the nasal dorsum, an incision of the skin was performed at the midline of the nasal dorsum to expose the naso-frontal and the nasomaxillary sutures. The bone lamella on the nasal dorsum was vertically weakened (dimensions of 3.5 × 0.5 cm) with the connecting line of the medial eyelid edge as the dorsal margin by using a 1.2 mm diamond drill (Proxxon micromot 50/E, Niersbach, Germany). A rhinotomy was subsequently performed by using a 3 mm chisel. The bone lamella was removed totally and placed into a moist compress for later re-implantation.

Afterwards, the cartilaginous septum was exposed by bluntly dissecting the perichondrium and forming bilateral mucoperichondrial flaps. The cartilaginous septum was removed leaving only 5 mm of the anterior and 5 mm of the posterior cartilaginous septum in place. The removed septum was used as a template to cut the scaffolds or the auricular cartilage in exactly the same dimensions. Subsequently, a chondrocyte-seeded decellularized xenogenic cartilage or a non-seeded decellularized xenogenic cartilage (study groups 1 and 2) or native auricular cartilage (control group 1) harvested from the right auricle of the rabbit was placed between the two mucosa flaps. In control group 2, the septum was removed with no septal replacement. The nasal bone was replaced and fixed by suturing the perichondrium with Vicryl 4-0. The incision was closed with Vicryl 5-0 intradermally. The operation procedure is demonstrated in Fig. 2 and the various implants prior to implantation are shown in Fig. 3.

Subcutaneous implantation on the back. Four non-seeded or seeded decellularized xenogenic cartilage discs of $1\ cm^2 \times 1$ mm in size (study groups 1 and 2) or four autologous ear cartilage discs ($1\ cm^2 \times 1$ mm) harvested from the right auricle (control group 2) were implanted via four paravertebral incisions. In order to provide protection against later manipulation of the rabbits, the wound suture was carried out intracutaneously. As given in international standard DIN EN ISO 10993-6 (Biological evaluation of medical devices - Part 6: Tests for local effects after implantation (ISO 10993-6:2007), German version EN ISO 10993-6:2009), the suture was placed at a minimal distance of 1.5 cm to the implanted disc in order to exclude interference.

2.4. Evaluation parameters

The behavior and wound healing of the rabbits was assessed macroscopically, after implantation, on a daily basis over an observation period of two weeks and weekly afterwards over the whole observation period of three months. After three or six months, the animals were anaesthetized with an infusion of 40 mg/kg ketamine and 4 mg/kg xylazine i.v. and were then killed with 2 mmol/kg potassium chloride KG i.v. The formation of the implanted neoseptum and form stability were then evaluated by Magnetic Resonance Imaging (MRI). In order to minimize, as far as possible, the formation of artifacts in the further course of the evaluation, the septa were not explanted; rather, the whole rabbit skull was skinned and decalcified over six weeks by using 0.7M ethylenediamine tetra-acetate (EDTA) in 4% Formalin pH 7.4. The EDTA-solution was changed every second day. Thereafter, the preparations were coronally sliced, attached to large specimen slides and stained. Cartilage formation, septal integrity and biocompatibility were evaluated histologically. The formation of newly synthesized cartilage extracellular matrix (ECM) was evaluated immunohistochemically.

MRI analysis. After the animals were killed after an observation period of three or six months, implants were examined by means of 7-Tesla MRI before explantation. Thus, we were able to evaluate the regenerated nasal septa regardless of cutting or staining artifacts. The rabbit heads were scanned in a whole-body 3T MRI system (Achieva 3T, Philips Healthcare, Best, The Netherlands) with a dedicated 8-channel knee coil positioned at the magnet's isocentre. All data were acquired at an isotropic spatial resolution of $0.4^3 mm^3$ with a T1-weighted turbo spinecho technique. Echo (TE) and repetition time (TR) were: TE/TR = 11 ms/337 ms acquiring eleven spin echoes after each excitation. MRIs were examined and evaluated by using OsiriX 6.0.1 (Pixmeo SARL, Bernex, Switzerland) and OS X Yosemite 10.10.1 (Apple Inc., Cupertino, USA). The length of the septum was measured to assess shrinkage after implantation. The thickness of the septum (implant + mucosa) was determined at

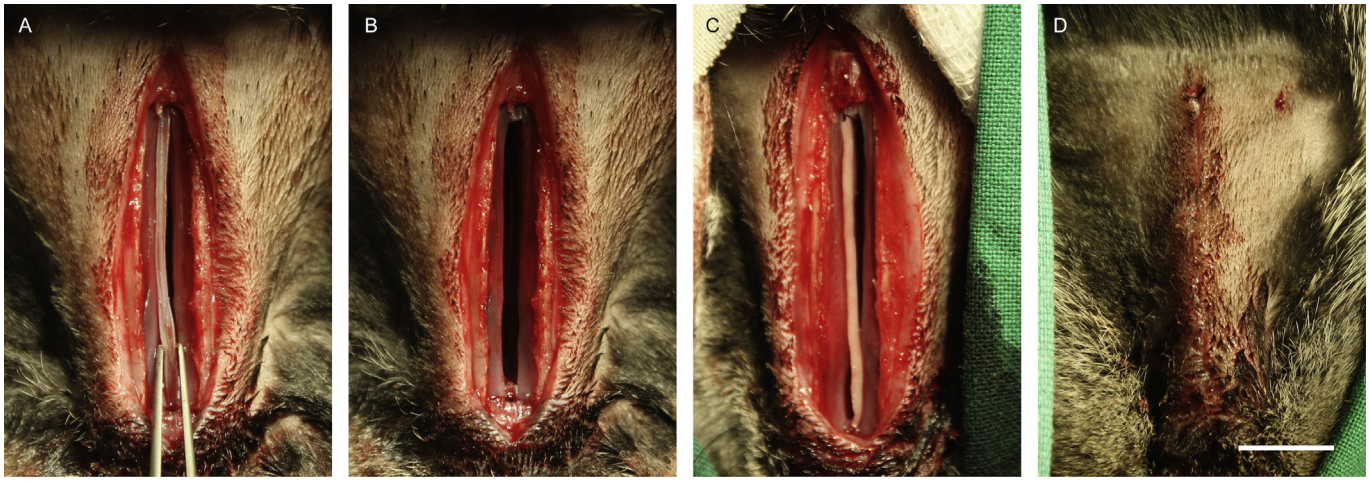

Fig. 2. Explantation of the native septum and implantation of a replacement material. A: the cartilage septum was exposed by dorsal rhinotomy. B: empty defect after removal of the septum. C: decellularized cartilage in the septal defect. D: Wound closure was performed intradermally. Index = 1 cm.

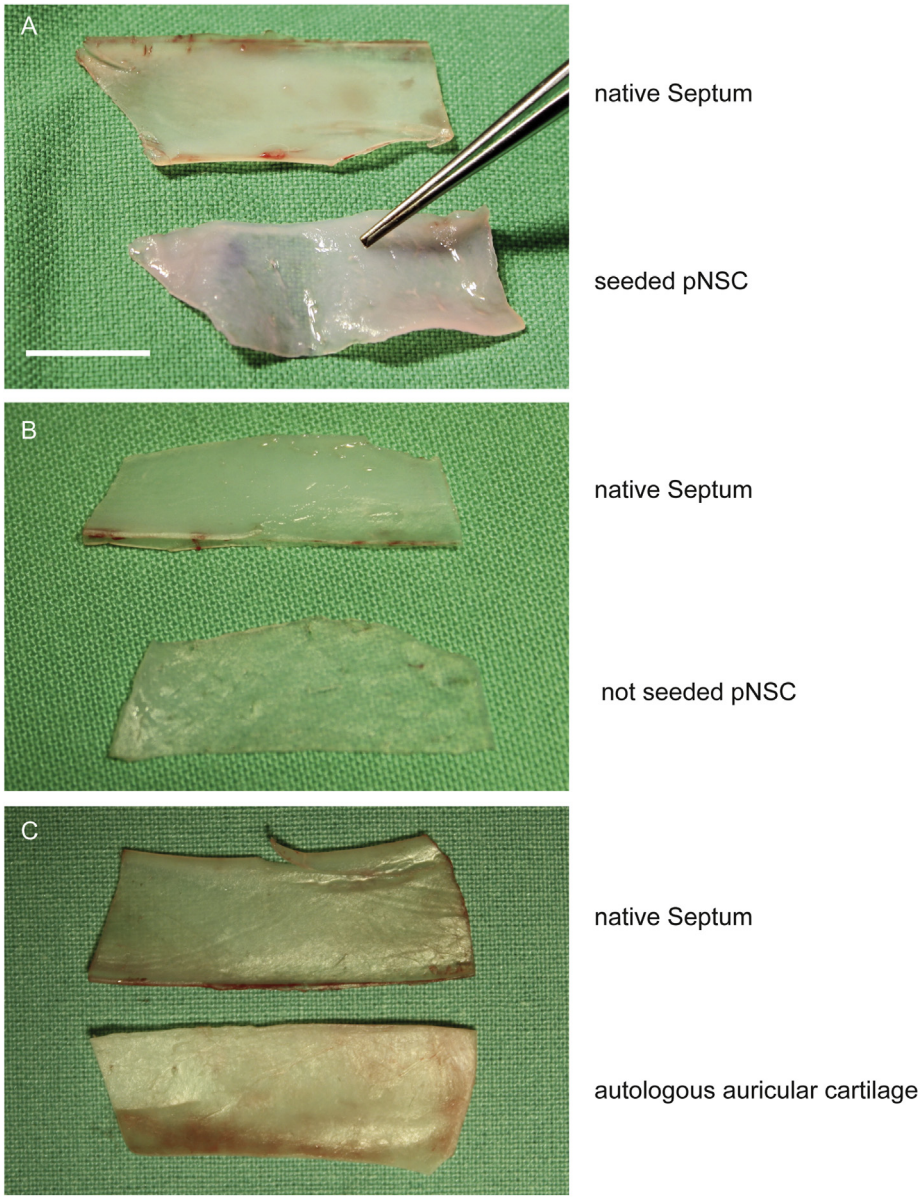

Fig. 3. Septal implants. A: native septum and seeded decellularized cartilage. B: native septum and decellularized cartilage. C: native septum and auricular cartilage. Index = 1 cm.

six points equally distributed from anterior to posterior of the newly implanted septum and a mean was calculated. The deviation of the septum was determined in degrees. The presence of a septum perforation was evaluated and if necessary measured. Any potential swelling of the conchae was analyzed. The swelling was scored 0 if not present, 1 if slightly present and the airway still filled with air, 2 if moderate with air-filled airways and 3 when the airway was fully blocked.

Histology and immunohistochemistry. Paraffin sections (3–5 μm) were prepared for histological and immunohistochemical evaluation. Sections were heat-fixed in the oven at 56 °C for 24h. Prior to being stained, the sections were rehydrated by using a decreasing alcohol series starting with xylol. Cell morphology, cell distribution and the presence of GAGs was assessed by haematoxylin and eosin (H&E) and Alcian blue staining.

For immunohistochemical detection of collagen type II, deparaffinized and rehydrated sections were digested with 1% hyaluronidase (Sigma, H3506-100 MG; in phosphate buffered saline (PBS)) and incubated for 15min at 37 °C. Subsequently, sections were rinsed with TBST buffer and digested with 0.2% pronase (Calbiochem, in PBS) for another 15min at 37 °C. All solutions for blocking and immunohistochemical staining were purchased from Dako and used according to the manufacturer's instructions (Envision + System-HRP, Dako). Primary antibody for collagen type II (II-II6B3; Developmental Studies Hybridoma Bank, isotype mouse IgG1) was added and incubated with the sections for 1h at room temperature. For aggrecan staining, sections were incubated in 0.5 U mL^{-1} chondroitinase ABC (Sigma–Aldrich) in PBS for 30min at room temperature to facilitate antibody access. The subsequent procedures were performed following the manufacturer's instructions (LSAB + System-HRP, Dako). Slides were counterstained with Meyer's haematoxylin.

Evaluation of biocompatibility. For the determination of the *in vivo* biocompatibility of the decellularized xenogenic cartilage scaffolds, H&E stained sections were evaluated microscopically according to DIN EN ISO 10993-6 (Biological evaluation of medical devices - Part 6: Tests for local effects after implantation (ISO 10993-6:2007); German version EN ISO 10993-6:2009). The biocompatibility evaluation was done for both, the intranasal and the subcutaneous implanted scaffolds. Histological characteristics such as encapsulation, inflammatory reactions, necrosis, scaffold degradation and the presence of polymorphous nuclear and giant cells were examined in the semi-quantitative evaluation system.

Statistical evaluation. Statistical analysis was performed by using GraphPad Prism (version 6.0b for Mac OS X, GraphPad Software, La Jolla, USA). The Kruskal–Wallis one way analysis of variance on ranks was used for evaluation of *in vitro* cytotoxicity tests.

3. Results

3.1. Cell shape and vitality during in vitro culture

During amplification, chondrocytes exhibited their typical pattern of dedifferentiation. Following cell seeding on the biomaterial construct and further 3D-cultivation for 14 days, the cells underwent redifferentiation regaining an almost round, cartilage-specific shape with deposition of ECM, as could be shown by Alcian staining and immunohistochemical staining for the cartilage-specific markers aggrecan and collagen II.

3.2. Macroscopic evaluation

In all animals, all wounds were closed with no wound infection or other complications within 10 days. Hair growth was normal in the operation field. No animal exhibited epistaxis or rhinorrhoea. In one animal of study group 1, two animals of study group 2, two animals of control group 1 and all animals in control group 2, an inspiratory whistling was heard in the first three days postoperatively. Oxygen saturation (SpO2) was always and in all animals > 98%.

3.3. MRI analysis

In the MRI evaluation the length of the septa, the mean thickness of the septa (implant + mucosa) and the deviation of the septa were determined in millimeters and degrees (Table 1). The presence of a septum perforation was evaluated and if necessary measured. Any potential swelling of the conchae was analyzed as explained in Materials and Methods.

The lengths of all septa showed no significant differences and were thus approximately the same in study group 1 with autologous seeded decellularized xenogenic cartilage as septal replacement, in study group 2 with non-seeded decellularized xenogenic cartilage as septal replacement, in control group 1 with autologous auricular cartilage as septal replacement and in control group 2 with removal of the septum and no septal replacement. The same applied to the mean thickness of the septa. Native animals in our study displayed a septum (including mucosa) with a thickness of 1.4 ± 0.6 mm. We identified a thickness of 1.3–1.4 mm after three months and 1.4–1.5 mm after six months among the study groups. In the control groups we found a thickness of 1.4–1.6 mm after three months and 1.1–1.2 mm after six months. In study groups 1 and 2, a pronounced deviation was detected after three months in both the horizontal and vertical directions of up to 3.3 mm deviation from the median (in the vertical direction, a maximum of 51.4°

Table 1
Results of MRI evaluation.

group	native	SG 1		SG 2		CG 1		CG 2	
Evaluation		3 months	6 months	3 months	6 months	3 months	6 months	3 months	6 months
septum length (mm)	48.5 ± 2.1	48.7 ± 1.2	48,5 ± 2.1	45.7 ± 4.2	52 ± 4.6	49.3 ± 1.5	50 ± 1	46.3 ± 3.5	51.3 ± 3.2
septum thickness (mm)	1.4 ± 0.6	1.3 ± 0.4	1.5 ± 0.6	1.4 ± 0.4	1.4 ± 0.5	1.6 ± 0.6	1.2 ± 0.5	1.4 ± 0.4	1.1 ± 0.3
septum deviation (mm)	0.2 ± 0.2	2.3 ± 1	0.5 ± 0.7	2.3 ± 0.7	0.9 ± 0.5	1.4 ± 0.4	1 ± 0.9	1.6 ± 0.8	0.8 ± 0.8
vertical (°)	5.5 ± 7.8	48.3 ± 3.1	7 ± 9.9	39 ± 9.5	26.7 ± 11.7	20.3 ± 9.8	18 ± 5.3	35.7 ± 12.9	18 ± 15.6
horizontal (°)	7.5 ± 10.6	34.7 ± 6.8	4 ± 5.7	29.3 ± 2.5	18.3 ± 4.2	18.6 ± 0.5	25.3 ± 18	25 ± 5.3	13.7 ± 18
septum perforation (mm)	-	-	-	-	0.1 × 1.6 0.2 × 0.4 -	-	-	1.9 × 1.2 0.8 × 0.7 0.6 × 1.6	6.7 × 6.3 - -
swelling	0/1	0/0/0	1/1	0/0/0	1/0/0	0/0/0	0/0/0	0/0/0	0/0/0

SG1 = study group 1 with seeded septal implants, SG2 = study group 2 with non-seeded septal implants, CG1 = control group 1 with septal implantation of autologous ear cartilage, CG2 = control group 2 with explantation of the septum and no replacement. Septum thickness = mean thickness of the septum (implant + mucosa) in millimeters. The swelling was scored 0 if not present, 1 if slightly and the airway still filled with air, 2 if moderate with air filled airways and 3 when the airway was fully blocked.

in study group 1 and 48.4° in study group 2/in the horizontal direction, a maximum of 41.5° in study group 1 und 31.8° in study group 2). Deviation declined over time and approached approximately the value of native septa after six months in study group 1.

In study group 2 without autologous seeding of the septum implants, septum perforations (0.1 × 1.6 mm and 0.2 × 0.4 mm) were observed in two animals after six months. After three months, none of the animals exhibited septum perforation. In study group 1, no septum perforation was found after three months or after six months. Further perforations were found in control group 2 with no septal replacement. After three months, all operated animals of this group showed septum perforation (1.9 × 1.2 mm, 0.8 × 0.7 mm and 0.6 × 1.6 mm). After six months, a large perforation of 6.7 × 6.3 mm was seen in one of the animals. Fig. 4 shows representative MRI images of the implants compared with a native control.

3.4. Histology

Large vital areas with cartilage-like tissue containing a significant amount of collagen II, aggrecan and sulphated GAGs, as shown both in the Alcian staining and immunohistochemically, were found in the seeded implants after three and six months. After three months, more cartilage-like tissue could be found in study group 1 compared with the six-month animals (Figs. 5 and 6). The degradation process of the initially developed neocartilage was more pronounced in study group 1 with autologous seeded implants compared to study group 2 with non-seeded implants. Furthermore an ingrowth of perichondrium could be found in study group 1 (Fig. 6).

The seeded group (study group 1) exhibited cartilage formation similar to native cartilage (Fig. 7). After 6 months the scaffold in the seeded group appears thicker than in the study group 2 and in the control groups. Newly ingrown cartilage could also be found in study group 2 with non-seeded implants. The newly synthesized cartilage was composed partly of perichondrial origin and partly of the remains of the septum cartilage (Fig. 8). Cartilage regeneration emanating from the perichondrium was not as homogenous as from the seeded scaffold. In control group 1 with native ear cartilage, degeneration took place in some areas of the transplant in two animals after six months. In control group 2 with no replacement

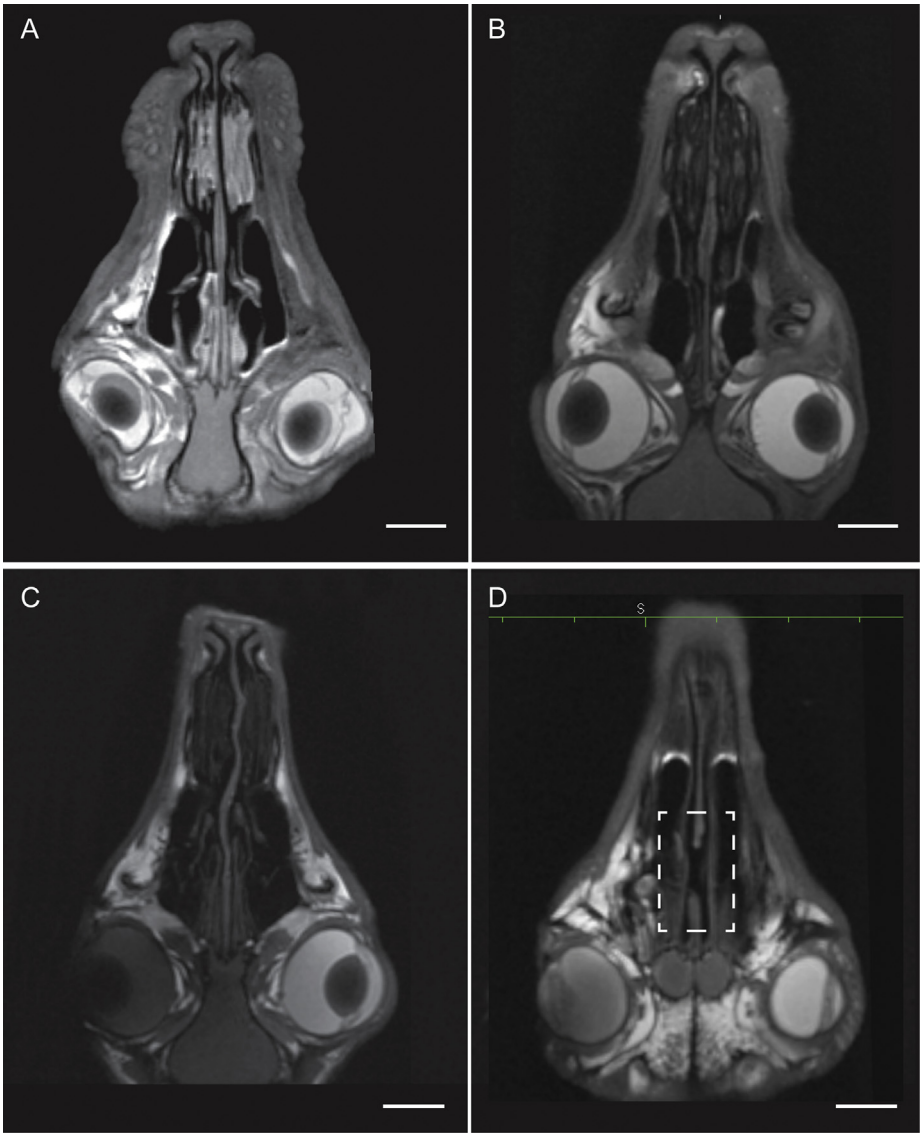

Fig. 4. 7-Tesla MRI of the septal implants after 6 months. A: native cartilage. B: decellularized cartilage. C: seeded decellularized cartilage with septal deviation. D: septal defect without replacement with septal perforation. Index = 1 cm.

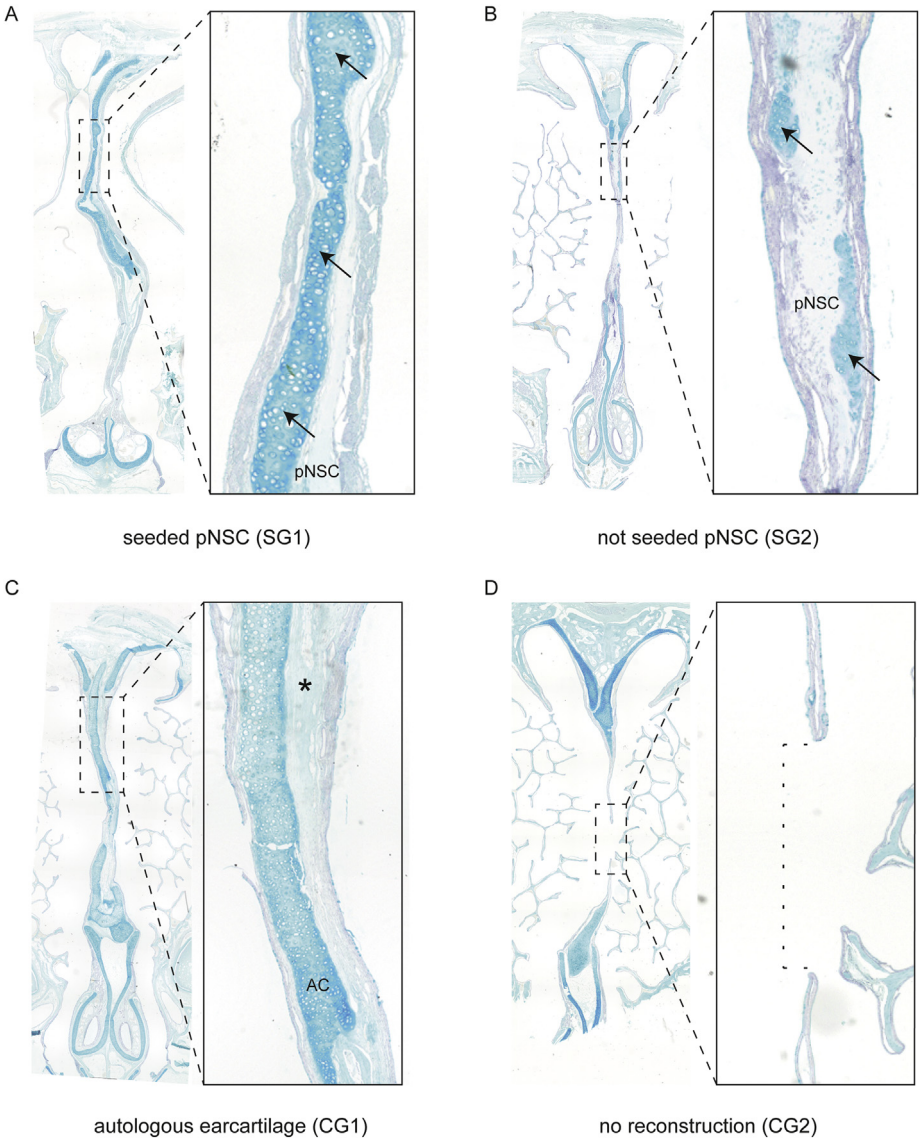

Fig. 5. Histological findings after 3 months. A: chondrocyte seeded decellularized xenogenic cartilage (SG1) with large areas of neocartilage (only minor cell infiltration, neocartilage is mainly present in the peripheral zones); B: non-seeded decellularized xenogenic cartilage (SG2) with areas of neocartilage; C: autologous native auricular cartilage (CG1); D: removal of the septum with no replacement (CG2). pNSC = decellularized xenogenic cartilage. Arrow = Neocartilage. * = fibrosis. AC = autologous auricular cartilage. Dotted line in D marks the septum perforation.

cartilage, we observed regeneration starting from the remains of the septum.

Good superficial integration could be observed, but hardly any infiltration. There was no deep infiltration of the scaffold, but good biocompatibility (see section 3.5) as superficial bonding was visible.

All implanted decellularized cartilage scaffolds maintained their shape during the whole observation period. The mucosa was attached tightly to the implant and surrounding tissue. After three months, one epithelialized septal perforation was seen in control group 2. After six months, one large epithelialized perforation could be found in control group 2 without septal replacement.

3.5. Evaluation of biodegradation and biocompatibility

Biocompatibility (BI) was evaluated in H&E staining after three and six months in all study and control groups. Fig. 9 shows histopathological biocompatibility evaluation.

After three months, slight infiltration with lymphocytes was detected in both study groups. Macrophages were only seen in one animal of study group 1 with a seeded implant. Plasma cells, giant cells, polymorphonuclear cells and necrosis were detectable in none of the sections. Fibroplasia and fibrosis were seen in study group 1 and 2 and in control group 1, but not in control group 2. A fatty infiltrate was seen in one animal of study group 2 and the control group 1. Degradation, degeneration or encapsulation was never seen. With regard to BI, the intranasal seeded implants caused slight irritation, the intranasal non-seeded implants caused no to slight irritation and native ear cartilage caused no irritation. A significant difference was noted between the BI of the seeded and the native ear cartilage groups. In subcutaneous placement, we determined slight to moderate irritation in the seeded group, moderate irritation in the non-seeded group and no irritation in the native group. A significant difference was seen between the non-seeded and the native implant.

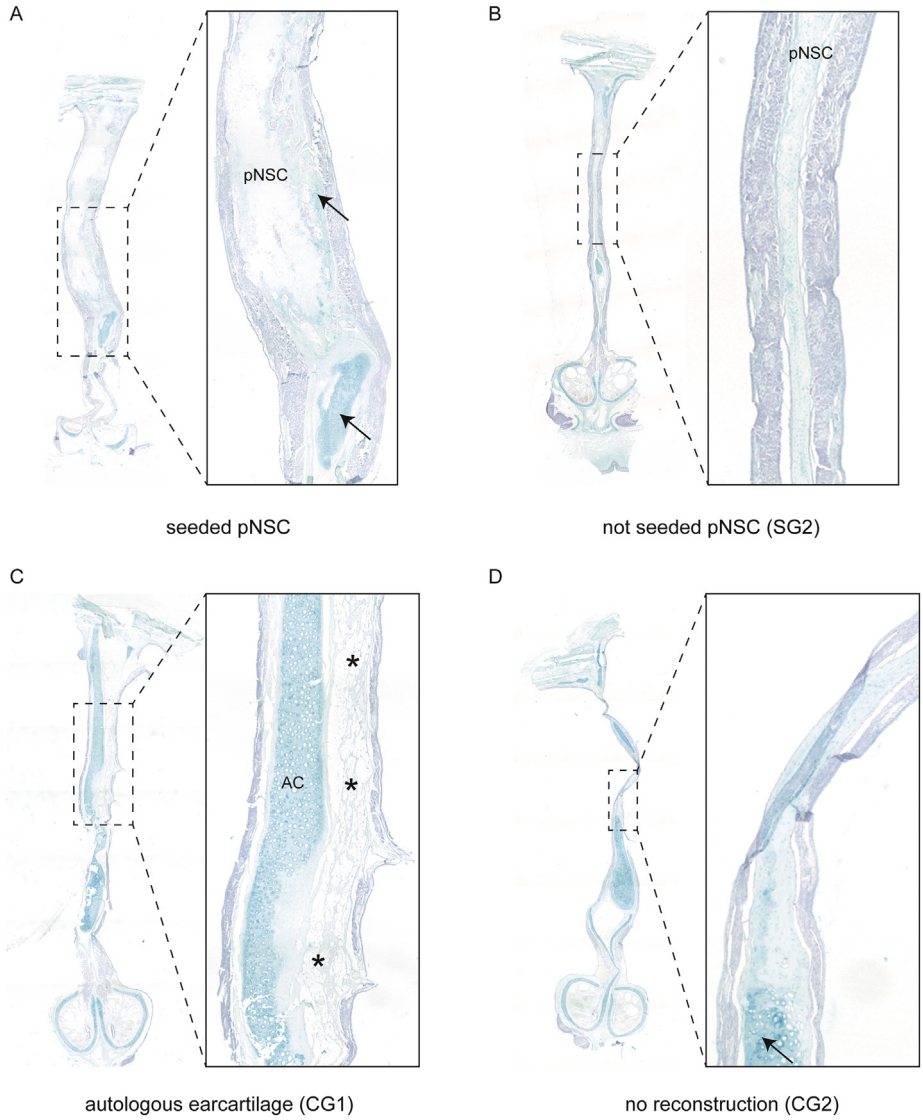

Fig. 6. Histological findings after 6 months. Both the study group and control group showed less cartilage tissue after 6 month than after 3 months. A: chondrocyte seeded decellularized xenogenic cartilage (SG1) with areas of neocartilage (SG1 showed less neocartilage after 6 months than after 3 month), ingrowth of perichondrium; B: non-seeded decellularized xenogenic cartilage (SG2) with areas of neocartilage, the degradation process of the initially developed neocartilage was more pronounced in SG1 compared with SG2; C: autologous native auricular cartilage (CG1); D: removal of the septum with no replacement (CG2). pNSC = decellularized xenogenic cartilage. Arrow = Neocartilage. * = fibrosis. AC = autologous auricular cartilage.

After six months, slight infiltration with lymphocytes was detected in both study groups. Macrophages were seen in one animal of study group 2, two animals of study group 1 and one animal of control group 1. In none of the sections did we detect plasma cells, giant cells or necrosis. Slight to moderate numbers of polymorphonuclear cells were seen in study group 1 and 2. Slight fibroplasia and fibrosis were observed in all animals of all study and control groups. A fatty infiltrate was seen in none of the section. Degradation, degeneration or encapsulation was never seen. Based on these results, the BI was examined for each group (Fig. 10 and Table 2). Intranasal seeded implants resulted in slight to moderate irritation, intranasal non-seeded implants no to moderate irritation and native ear cartilage no to slight irritation. No significant difference was found. In subcutaneous placement, moderately irritation was found in the seeded, slight to marked irritation in the non-seeded and no irritation in the native group. A significant difference was noted between the seeded and the native implant.

Overall, the mean BI of all implants was evaluated as showing moderate irritation.

4. Discussion

In this study, we have examined the long-term stability and BI of decellularized xenogenic cartilage as a matrix for the regeneration and cultivation of cartilage by using tissue engineering. This decellularized cartilage matrix had been developed and patented in Erlangen and had shown very promising results in vitro and in a rat model (Elsaesser et al., 2014). In order to further examine the material and to determine its clinical applicability, we tested its BI according to DIN EN ISO 10993-6, Appendix E, by using subcutaneous implantation and a clinically oriented septal rabbit model. For this purpose, a new rat model (Bermueller et al., 2013) was first modified and then scaled to the rabbit in order for us to be able to examine larger constructs with dimensions of clinical relevance. The nasal septum was of particular interest as an implantation site since the implant was not covered by copious soft tissue, but by a thin layer of perichondrium and mucosa. Therefore, the risk of wound healing disorders and septal perforations was deemed to be high. We used an autologous rabbit model as employed in

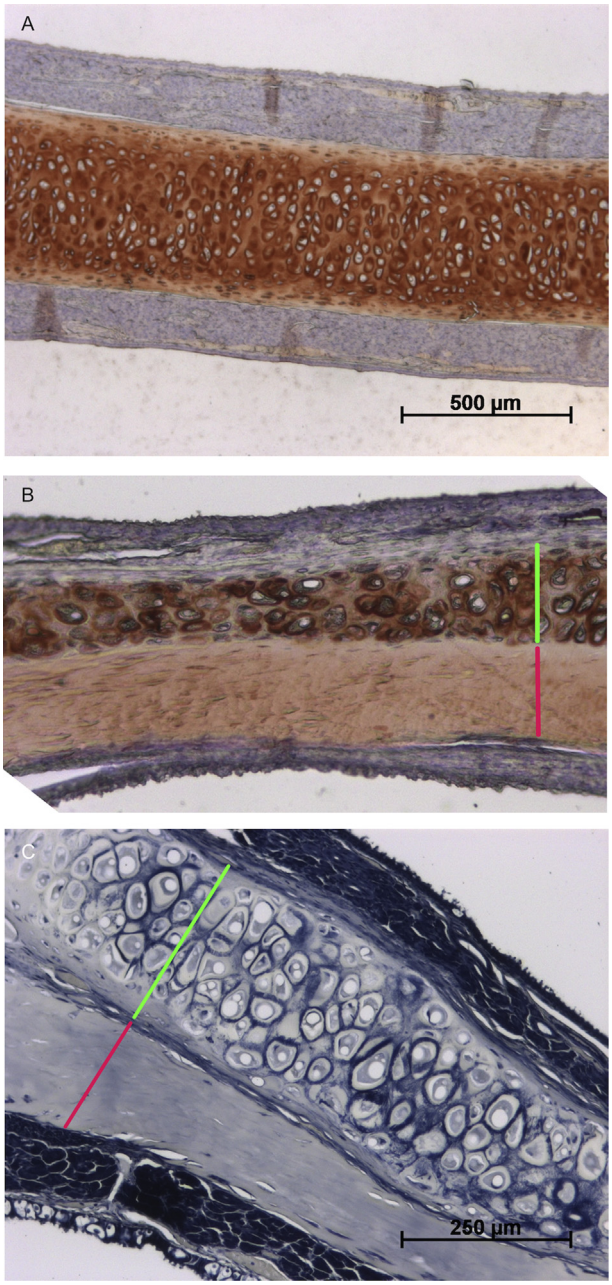

Fig. 7. Immune histology of seeded decellularized cartilage implant. A: native septum, anti-collagene II stain. B: seeded decellularized cartilage after 3 months, cartilage formation similar to native cartilage, anti-collagene II stain. C: seeded decellularized cartilage after 3 months, cartilage formation similar to native cartilage, Verhoeff stain. Red bar = pNSC. Green bar = Neocartilage.

preliminary studies (von Bomhard et al., 2013) in order to be able to take immunological reactions into consideration.

When selecting the animal model, we have chosen Chinchilla Bastard rabbits which is a wild type and know to be immunocompetent, because we wanted to work with an autologous model. Various animal models for the examination of different implants such as Polydioxanon (PDS) or autologous implants for the reconstruction of nasal septa have been described (Verwoerd et al., 1991; Boenisch et al., 2003). Martinez and Dolci used a similar model to test whether bacterial cellulose can be used to prevent septal perforations in nasal surgery (Martinez Neto and Dolci, 2010). However, animals were operated transnasally instead of dorsal rhinotomy being performed. This method is less traumatic but, in our opinion, has two disadvantages: first, only small defects can be analyzed (in this study 7 × 7 mm) and second, the overview is significantly reduced. Kaiser et al. performed dorsal rhinotomy to remove the septum subtotally without replacement and to examine regeneration processes (Kaiser et al., 2006). They chose a laterally based skin/periosteal flap to access the osseous nasal dorsum in order not to place the suture directly over the osseous defect afterwards. Therefore we chose this approach to the nasal septum.

Mola et al. also chose dorsal rhinotomy to access the nasal septum of the rabbit. They placed Alloderm, Dacron, Gore-Tex and cartilage in four septal defects. Alloderm is carcass tissue that has been stripped of its cellular components by means of various processing steps, leaving the basic mesenchymal structure. It has been described as highly advantageous in comparison with the synthetic materials Gore-Tex and Dacron as it shows no inflammatory response, no encapsulation and good histocompatibility (Mola et al., 2007). Alloderm was used by Kridel et al. in a clinical study in 1998 in order to repair septal perforation. For 11 out of 12 patients, this was found to be successful (Kridel et al., 1998). Ayshford et al. were able to close 13 out of 17 septal defects by using Alloderm (Ayshford et al., 2003). Cohen and Mirza have used Alloderm in order to close mucosal defects (Cohen and Mirza, 2000). However, as a matrix for the cultivation of cartilage, materials such as Alloderm do not anatomically and biomechanically correspond to the native tissue. Our goal was to design the scaffold as physiological as possible. Both the histopathological structure and the form stability of the material should ideally mimic the native tissue. Moreover, the scaffold should support and facilitate the attachment, proliferation and differentiation of chondrocytes. We created an extracellular cartilage matrix that fulfils these requirements and provides an ideal physiological niche for the desired cell type.

To obtain a sufficient number of cells for seeding large-scale three-dimensional scaffolds involving the use of primary chondrocytes, a cell amplification following an autologous tissue biopsy needs to be performed. During this expansion phase, chondrocytes dedifferentiate and lose their phenotype (Darling and Athanasiou, 2005). After retransfer into a 3D cell complex, cells redifferentiate, but they do not completely regain their original phenotype (Buschmann et al., 1992).

We applied autologous auricular chondrocytes for nasal cartilage regeneration. Henderson et al. found differences in cell proliferation rates, extracellular matrix production and gene expression between auricular and nasoseptal chondrocytes. Auricular chondrocytes proliferated less and more slowly than nasoseptal chondrocytes. However, auricular chondrocytes produced more cartilage-like matrix than nasoseptal chondrocytes. Auricular chondrocytes showed a higher expression of anabolic growth factors such as BMP5 and IGF1, which could be a possible explanation for the observed higher matrix production by auricular chondrocytes (Hellingman et al., 2011). Isogai evaluated different bovine chondrocytes for use in tissue engineering. He also found differences in cell proliferation rates, extracellular matrix production and type II collagen and aggrecan gene expression. For both genes, higher expression was found for nasoseptal cells compared to auricular cells (Isogai et al., 2006). Despite these differences, we chose auricular chondrocytes to grow nasal cartilage because auricular cartilage is easier to biopsy, more accessible and larger biopsies are possible.

The xenogenic scaffolds from study group 1 and 2 were preoperatively cut to size by using a microtome to match the thickness of native rabbit septa. Intraoperatively, all implants were adjusted to the exact size of the explanted cartilage septum. Because of the exact sizing of the septum implants, fixation was no longer necessary after implantation. The advantage of this was that the

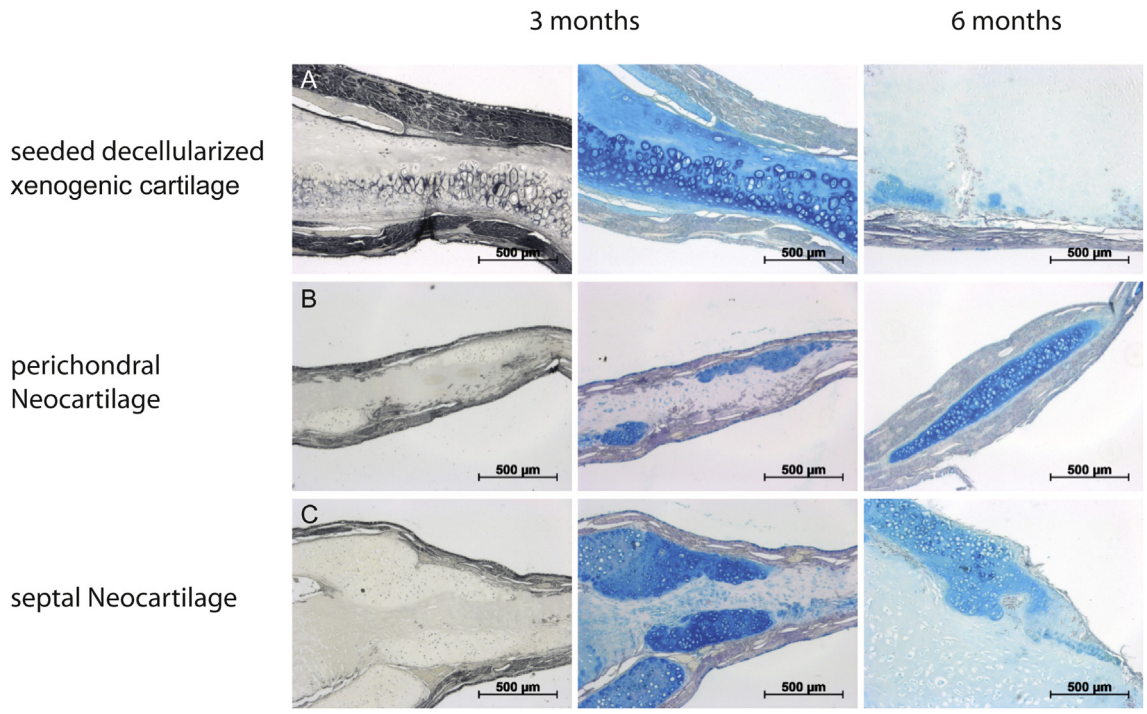

Fig. 8. Origin of neocartilage formation. A: seeded decellularized xenogenic cartilage. B: non-seeded decellularized xenogenic cartilage, perichondral neocartilage. C: non-seeded decellularized xenogenic cartilage, septal neocartilage.

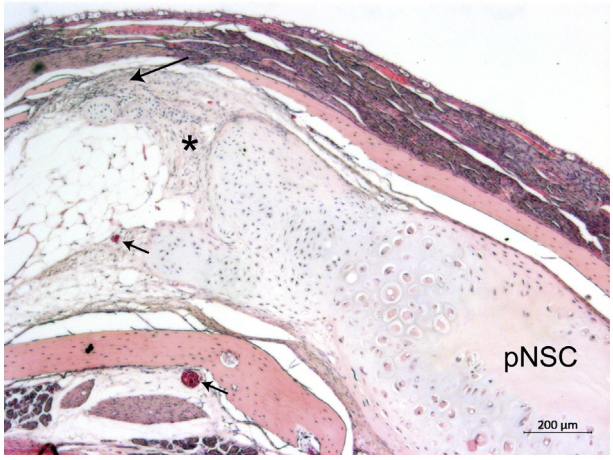

Fig. 9. Histopathological biocompatibility evaluation. * = fibrosis. Long arrow = Lymphocytes. Short arrow = blood vessels. pNSC = decellularized xenogenic cartilage.

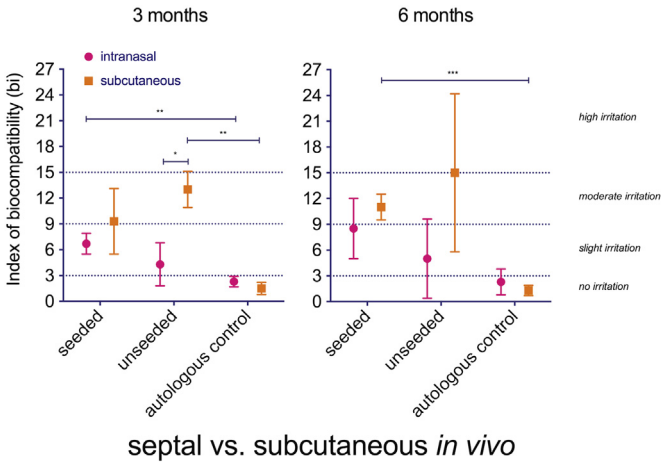

Fig. 10. Biocompatibility of the evaluated implants in septal vs. subcutaneous placement after 3 and 6 months.

implants could be evaluated for BI without the disruptive influence of sutures or other fixation material. No dislocation of implants was found during the entire observation period or during follow-up examinations. The use of septum foil has been described to avoid the swelling of operated nasal septa and to prevent septal perforations (Tweedie et al., 2010). Whether septum foil also has an effect on BI evaluation was unclear and we therefore decided not to use it.

The wounds of all operated animals healed without complications within 10 days. One animal from study group 1, two animals from study group 2, two animals from control group 1 and all animals from control group 2 displayed postoperative inspiratory whistling lasting a maximum of three days but this did not impair the behaviour of the animals. This noise was most likely attributable to septal instability (especially in control group 2) and/or mucosal swelling. Despite the use of allogeneic non-vascularized implants and the operation of the airways, which are populated with microbes, no infections were observed.

The histological stains showed large areas with vital cartilage-like tissue and a significant amount of collagen type II, aggrecan and sulphated GAGs in study group 1 with seeded xenogenic septum implants after three and six months. The newly synthesized cartilage was mainly present in the peripheral zones of the seeded 3D implants. We observed similar results in previous studies (von Bomhard et al., 2013). Because of the high density and low porosity the center of the seeded decellularized xenogenic cartilage could not be repopulated and an ingrowth of cells was rarely found. Additionally in the center of the biomaterial, the nutrient supply,

Table 2
Evaluation of biodegradation and biocompatibility.

	Group	Month		PNCs	Lympho-cytes	Plasma cells	Macro-phages	Giant cells	Necrosis	Fibro-plasia	Fibrosis	Fatty infiltrate
Intranasal placement	Seeded	3	Median	0	1	0	0	0	0	2	2	0
			± MD	0.00	0.00	0.00	0.58	0.00	0.00	0.58	0.00	0.58
		6	Median	1	1	0	2	0	0	2	1	0
			± MD	0.00	0.71	0.00	0.71	0.00	0.00	0.71	0.00	0.00
	Non-seeded	3	Median	0	1	0	0	0	0	1	1	0
			± MD	0.00	1.00	0.00	0.00	0.00	0.00	0.00	0.58	0.00
		6	Median	1	0	0	0	0	0	1	1	0
			± MD	1.00	0.58	0.00	0.58	0.00	0.00	0.58	0.00	0.00
	Native cartilage	3	Median	0	0	0	0	0	0	1	1	0
			± MD	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.58
		6	Median	0	0	0	0	0	0	1	1	0
			± MD	0.00	0.00	0.00	0.58	0.00	0.00	0.58	0.00	0.00
	Perforation control	3	Median	0	0	0	0	0	0	0	0	0
			± MD	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
		6	Median	0	0	0	0	0	0	1	1	0
			± MD	0.00	0.00	0.00	0.00	0.00	0.00	0.58	0.00	0.00
Subcutaneous placement	Seeded	3	Median	2	0	0	1	0	1	1	2	0
			± MD	1.53	0.00	0.00	1.00	0.00	0.58	0.00	0.58	0.00
		6	Median	1	1	0	1	1	0	1	2	0
			± MD	0.58	0.00	0.00	0.58	0.58	0.00	0.58	0.00	0.00
	Non-seeded	3	Median	3	0	0	2	0	1	1	2	0
			± MD	0.58	0.00	0.00	0.58	0.00	0.58	0.00	0.58	0.00
		6	Median	2	1	0	2	1	1	1	2	0
			± MD	1.41	1.41	0.00	0.71	0.00	0.71	0.00	0.71	0.00
	Native cartilage	3	Median	0	0	0	0	0	0	1	1	0
			± MD	0.00	0.00	0.00	0.00	0.00	0.00	0.71	0.00	0.00
		6	Median	0	0	0	0	0	0	0	1	0
			± MD	0.00	0.00	0.00	0.00	0.00	0.00	0.58	0.58	0.58

Summarized classification scores of seeded and non-seeded scaffolds: 3 and 6 months after implantation compared with the native cartilage group (0 = absent, 1 = slight, 2 = moderate, 3 = marked, 4 = severe). MD, median deviation; PNCs, polymorphonuclear cells.

which exclusively takes place via diffusion in cartilage tissue, is significantly worse. In this study, the septal mucosa could not be sufficiently refixed to the implant because of the surgical technique chosen and the small dimensions of the rabbit airways. Moreover, the exact dimensions of the implant allowed no luxation to be carried out from the implant site. However, the partially non-attached mucosa led to a non-insignificant reduction in the nutrient supply. This is possibly an additional reason for the reduced presence of vital cartilage tissue in some areas. Even if only a little ingrowth of cells can be observed, we assume that a slow tissue remodeling occurs and the allogeneic cartilage will be transformed and replaced by autologous cartilage as cartilage tissue formation is not completed after 6 months. Alternatively, the formation of scar tissue would also be conceivable which, in our opinion, would also provide clinically tolerable results.

Indications have been obtained that cartilage can regenerate after traumatic loss in rabbits (Lopez Aguado et al., 1992; Kaiser et al., 2006), although according to current scientific opinion, cartilage is generally not able to heal ad integrum in most species. The perichondrium, which covers most cartilage structures in the head and neck area, can be involved in regeneration processes as a source of mesenchymal stem cells (Pountos and Giannoudis, 2005). Verwoed-Verhoef et al. have postulated that new cartilage tissue is formed only two weeks after submucosal cartilage resection and emanates from perichondrial stem cells (Verwoerd-Verhoef et al., 1998). Kaiser et al. have also found cartilage regeneration in seven-month old rabbits (Kaiser et al., 2006). In control group 2, we have also detected cartilage regeneration emanating from the remaining septum parts and from the perichondrium, both after three and after six months. Because rabbits show better cartilage regeneration than humans we recommend further studies in larger animal models or humans prior to a wide clinical introduction.

After a three-month period, both the study group and control group exhibited more cartilage tissue than after six months. Many papers report the same observations and name resorption processes as a possible cause (Rotter et al., 2005). Resorption is mainly transmitted through macrophages as part of the physiological tissue repair. Autoantibodies against parts of the cartilage tissue, such as collagen type IX and XI, trigger absorption of the transplant. This process even occurs when transplanting native autologous cartilage (Bujia et al., 1994; Naumann et al., 1994). Resorption processes occur when a structure is recognized as foreign due to a potential antigenic component. With the decellularization process we tried to eliminate this immunological component and showed in previous studies that we were able to remove autoantibodies and cellular components as far as possible (Schwarz et al., 2012b). Our goal was to remove all unfavourable characteristics of a xenogenic implant by decellularization. Compared with study group 2 with non-seeded implants, the absorption process was more pronounced in study group 1 with autologous seeded implants. This leads to the assumption that one finds more antigens in cultured tissues and the remains of the culture media (von Bomhard et al., 2013). Degradation of the developed cartilage was particularly pronounced in the seeded group and an ingrowth of perichondrium into these areas was observed. Thus, there was an ingrowth of orthotopic cells while the initial cell colonization degraded, which may be due to the heterotopic cell source as auricular chondrocytes were used. Furthermore the scaffold in the seeded group appears thicker than in the other groups after 6 months. This may be due to the cutting process, as a different microtome was used.

Regardless of histologically visible resorption processes, the MRI evaluation found a good form stability of the nasal septa. The size did not change significantly over the course of time, neither in study group 1 nor 2; even the thickness was rather constant. Wong et al. described the septal cartilage with a thickness of around 0.25–0.75 mm (Wong et al., 2001). Native animals in our study displayed a septum (including mucosa) with a thickness of

1.4 ± 0.6 mm. We identified a thickness of 1.3–1.4 mm after three months and 1.4–1.5 mm after six months among the study groups. Previously, implants in study group 1 and 2 were cut to a thickness of 350 μm with a microtome according to the native cartilaginous septum. Animals of control group 1 with autologous auricular cartilage as septal replacement exhibited a thickness of 1.6 mm after three months and 1.2 mm after six months. Initially, the septa of these animals were too thick but, after the process of shrinking was completed, their septa were too thin compared with native controls. Newly formed cartilage and scaffold were not distinguished by analysing the shrinkage because only the whole septum was visible in 7-Tesla MRI. Nasal septa of study group 1 were 4.3% v/v smaller after three months than the native controls; in study group 2, 7.9% v/v of septa were smaller and in control group 1 16.1% v/v larger. This was in particular because the autologous auricular cartilage was initially too thick, which in turn affected the total volume. After six months though, this was normalized because of the aforementioned shrinking process. Bruce et al. found shrinking of 23.1% by weight in the transplantation of autologous cartilage after three months (Lattyak et al., 2003). By evaluating MRIs over three to six months, we found equivalent shrinking of 26.9% v/v on average when transplanting autologous auricular cartilage (control group 1). After six months, implants in study group 1 were 5.4% v/v larger than in the native control, whereas implants in study group 2 were 7.0% v/v larger and 15.1% v/v smaller in the control group 1. Differences in study group 1 and 2 were not significant. However, the difference in control group 1 was significant. Our histological results support these findings. In continuative studies, repetitive MRI examinations are a further option, since this is a non-invasive examination, apart from the necessary anesthesia.

With subcutaneous implantation, the BI index of the applied materials revealed marginally more irritation than with septal implantation. This must be attributable to the immense immunological capacity of skin and its role regarding infection defense (Mangoni et al., 2016). Since materials are tested subcutaneously for approval according to DIN EN ISO 10993-6, Appendix E, they could well be considered for intranasal usage with good results. Among the non-seeded study group BI index of septal implantation was slightly more favorable than that among the seeded group. Interestingly, this effect was reversed in subcutaneous placement. Therefore, an autologous cell seeding is advantageous with regard to BI for subcutaneous, but not for nasal usage. However, autologously seeded implants did not exhibit septal perforation after both a three- and a six-month period. Study group 2 with non-seeded septal biomaterial displayed two small perforations (0.1 × 1.6 mm and 0.2 × 0.4 mm; 66%) after six months. This constituted a clear advantage of the seeded group, since none of the animals showed septal perforation over time. Thus, cell seeding derives a benefit regarding long-term stability. The presence of septum perforations is likely related to the stability of the material. Without cell seeding the material is much less stable, so the non-seeded material was more likely to lead to septum perforation when compared with the seeded material. Thus we have observed a few areas that could not be sufficiently stabilized. The perforations were also mainly seen in the marginal area or transition area, as this is the area with the least stability. We do not consider absorption to be a possible cause of perforation. We detected absorption in a few areas, but not in areas where we found perforations. However, animals that received an autologously seeded septum replacement presented more pronounced deviations of the septa. Nevertheless, these decreased over time. Further perforations could be found in control group 2 without septum replacement. After three months, all operated animals presented a septal perforation (1.9 × 1.2 mm, 0.8 × 0.7 mm and 0.6 × 1.6 mm). After six months, one of the animals suffered a large perforation of 6.7 × 6.3 mm.

Among control group 1 with native auricular cartilage as the septal replacement, two animals presented cartilage degeneration after six months. Overall, these results emphasize the importance of long-term observations, which, in most cases, cannot be achieved because of financial and time constraints.

5. Conclusion

This study analyzed the long-term outcome of spectrally implanted cartilage replacement materials. In addition to decellularized and autologously seeded xenogenic septa, non-seeded decellularized xenogenic septa and, in the control groups, autologous auricular cartilage (the current gold standard) and non-reconstructed septum defects were analyzed with regard to BI, form stability and clinical parameters.

Over the whole observation period, the BI index of the autologous auricular cartilage group (CG1) showed the best results. It was only slightly higher in the non-seeded and slightly higher than this in the seeded group (auricular cartilage < non-seeded < seeded). A significant difference between the native group and the seeded group was seen after three months. In subcutaneous implantation, the BI index was also most favorable in CG1. It was slightly higher in the seeded and higher again in the non-seeded group (auricular cartilage < seeded < non-seeded). A significant difference was seen between the native group and the seeded group, even after six months. All in all, the average BI index of all implants indicated moderate irritation.

MRI imaging demonstrated good form stability and long-term stability in all groups. The length of the septa was stable over time and deviations were only mild to moderate. Septum perforations could be observed after both a three- and a six-month period. After three months, perforations occurred only in all animals of the control group without septal replacement. After six months, two small septal perforations (0.1 × 1.6 and 0.2 × 0.3 mm) were found in one animal of the non-seeded study group, with a significantly larger perforation with 6.7 × 6.3 mm in CG2.

In summary, good BI without an excessive rejection reaction was demonstrated in all groups. In the study groups with or without cell seeding, no complete cartilage healing occurred ad integrum, whereas cartilage formation from the perichondrium was more irregular than from the seeded scaffold. However, long-term stable and reliable septal reconstruction was achieved in both groups. Because of the markedly lower effort required, the slightly better BI and the less pronounced septum deviations, non-seeded decellularized xenogenic septal cartilage is favoured for possible clinical application. Cell seeding is advantageous only with regard to septal perforations.

Conflicts of interest

None.

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by the University of Iowa, Department of Biological Sciences, Iowa City, IA 52242, USA.

References

Ayshford CA, Shykhon M, Uppal HS, Wake M: Endoscopic repair of nasal septal perforation with acellular human dermal allograft and an inferior turbinate flap. Clin Otolaryngol Allied Sci 28(1): 29–33, 2003

Benders KE, van Weeren PR, Badylak SF, Saris DB, Dhert WJ, Malda J: Extracellular matrix scaffolds for cartilage and bone regeneration. Trends Biotechnol 31(3): 169–176, 2013

Bermueller C, Schwarz S, Elsaesser AF, Sewing J, Baur N, von Bomhard A, et al: Marine collagen scaffolds for nasal cartilage repair: prevention of nasal septal perforations in a new orthotopic rat model using tissue engineering techniques. Tissue Eng Part A 19(19–20): 2201–2214, 2013

Boenisch M, Tamas H, Nolst Trenite GJ: Influence of polydioxanone foil on growing septal cartilage after surgery in an animal model: new aspects of cartilage healing and regeneration (preliminary results). Arch Facial Plast Surg 5(4): 316–319, 2003

Breiter R, Koerber L, Schwarz S: Collagen implant; 2010, EP Patent 2,236,544

Bujia J, Alsalameh S, Naumann A, Wilmes E, Sittinger M, Burmester GR: Humoral immune response against minor collagens type IX and XI in patients with cartilage graft resorption after reconstructive surgery. Ann Rheum Dis 53(4): 229–234, 1994

Buschmann MD, Gluzband YA, Grodzinsky AJ, Kimura JH, Hunziker EB: Chondrocytes in agarose culture synthesize a mechanically functional extracellular matrix. J Orthop Res 10(6): 745–758, 1992

Cohen NA, Mirza N: Acellular human dermal allograft in repair of unilateral partial-thickness and full-thickness nasal septal mucosal defects. Laryngoscope 110(12): 2005–2007, 2000

Darling EM, Athanasiou KA: Rapid phenotypic changes in passaged articular chondrocyte subpopulations. J Orthop Res 23(2): 425–432, 2005

Elsaesser AF, Bermueller C, Schwarz S, Koerber L, Breiter R, Rotter N: In vitro cytotoxicity and in vivo effects of a decellularized xenogeneic collagen scaffold in nasal cartilage repair. Tissue Eng Part A 20(11–12): 1668–1678, 2014

Haisch A, Duda GN, Schroeder D, Groger A, Gebert C, Leder K, et al: The morphology and biomechanical characteristics of subcutaneously implanted tissue-engineered human septal cartilage. Eur Arch Otorhinolaryngol 262(12): 993–997, 2005

Hellingman C, Verwiel E, Slagt I, Koevoet W, Poublon R, Nolst-Trenite G, et al: Differences in cartilage-forming capacity of expanded human chondrocytes from ear and nose and their gene expression profiles. Cell Transplant 20(6): 925–940, 2011

Isogai N, Kusuhara H, Ikada Y, Ohtani H, Jacquet R, Hillyer J, et al: Comparison of different chondrocytes for use in tissue engineering of cartilage model structures. Tissue Eng 12(4): 691–703, 2006

Kaiser ML, Karam AM, Sepehr A, Wong H, Liaw LH, Vokes DE, et al: Cartilage regeneration in the rabbit nasal septum. Laryngoscope 116(10): 1730–1734, 2006

Kridel RW, Foda H, Lunde KC: Septal perforation repair with acellular human dermal allograft. Arch Otolaryngol Head Neck Surg 124(1): 73–78, 1998

Langer R, Vacanti JP: Tissue engineering. Science 260(5110): 920–926, 1993

Lattyak BV, Maas CS, Sykes JM: Dorsal onlay cartilage autografts: comparing resorption in a rabbit model. Arch Facial Plast Surg 5(3): 240–243, 2003

Liu M, Zeng X, Ma C, Yi H, Ali Z, Mou X, et al: Injectable hydrogels for cartilage and bone tissue engineering. Bone Res 30(5): 17014, 2017

Lopez Aguado D, Monserrat JR, Perez Pinero B, Campos Banales ME, Gutierrez R, Diaz Flores L: Neochondrogenesis in the septal area after submucous cartilaginous resection. Acta Otolaryngol 112(3): 539–544, 1992

Mangoni ML, McDermott AM, Zasloff M: Antimicrobial peptides and wound healing: biological and therapeutic considerations. Exp Dermatol 25(3): 167–173, 2016

Martinez Neto EE, Dolci JE: Nasal septal perforation closure with bacterial cellulose in rabbits. Braz J Otorhinolaryngol 76(4): 442–449, 2010

Mola F, Keskin G, Ozturk M, Muezzinoglu B: The comparison of acellular dermal matric (Alloderm), Dacron, Gore-Tex, and autologous cartilage graft materials in an experimental animal model for nasal septal repair surgery. Am J Rhinol 21(3): 330–334, 2007

Morouco P, Ângelo D, Francisco L, Moura C, Alves N: Tissue engineering for temporomandibular joint disc repair and regeneration: a methodological perspective. Adv Cell Mol Otolaryngol 4(1): 33709, 2016

Naumann A, Bujia J, Hammer C, Wilmes E: Autoantibodies against cartilage components: clinical relevance for reconstructive surgery in the area of the head and neck. Laryngorhinootologie 73(5): 253–257, 1994

Pountos I, Giannoudis PV: Biology of mesenchymal stem cells. Injury 36(Suppl. 3): S8–S12, 2005

Rotter N, Bucheler M, Haisch A, Wollenberg B, Lang S: Cartilage tissue engineering using resorbable scaffolds. J Tissue Eng Regen Med 1(6): 411–416, 2007

Rotter N, Haisch A, Bucheler M: Cartilage and bone tissue engineering for reconstructive head and neck surgery. Eur Arch Otorhinolaryngol 262(7): 539–545, 2005

Schwarz S, Elsaesser AF, Koerber L, Goldberg-Bockhorn E, Seitz AM, Bermueller C, et al: Processed xenogenic cartilage as innovative biomatrix for cartilage tissue engineering: effects on chondrocyte differentiation and function. J Tissue Eng Regen Med 9(12): E239–E251, 2015 Dec

Schwarz S, Koerber L, Elsaesser AF, Goldberg-Bockhorn E, Seitz AM, Durselen L, et al: Decellularized cartilage matrix as a novel biomatrix for cartilage tissue-engineering applications. Tissue Eng Part A 18(21–22): 2195–2209, 2012b

Tweedie DJ, Lo S, Rowe-Jones JM: Reconstruction of the nasal septum using perforated and unperforated polydioxanone foil. Arch Facial Plast Surg 12(2): 106–113, 2010

Verwoerd CD, Verwoerd-Verhoef HL, Meeuwis CA, van der Heul RO: Wound healing of autologous implants in the nasal septal cartilage. ORL J Otorhinolaryngol Relat Spec 53(5): 310–314, 1991

Verwoerd-Verhoef HL, ten Koppel PG, van Osch GJ, Meeuwis CA, Verwoerd CD: Wound healing of cartilage structures in the head and neck region. Int J Pediatr Otorhinolaryngol 43(3): 241–251, 1998

von Bomhard A, Veit J, Bermueller C, Rotter N, Staudenmaier R, Storck K, et al: Prefabrication of 3D cartilage contructs: towards a tissue engineered auricle – a model tested in rabbits. PloS One 8(8): e71667, 2013

Wong BJ, Chao KK, Kim HK, Chu EA, Dao X, Gaon M, et al: The porcine and lagomorph septal cartilages: models for tissue engineering and morphologic cartilage research. Am J Rhinol 15(2): 109–116, 2001