

Review

Auricular Chondrocytes – From Benchwork to Clinical Applications

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Abstract. Auricular chondrocytes, obtained from human auricular cartilage, can be grown easily in culture and have been used as a source for autologous cell/tissue transplant in several fields of reconstructive surgery. In addition, auricular chondrocytes/cartilage are being increasingly used for tissue engineering approaches to create artificial organs. Moreover, auricular chondrocytes have been used to improve biocompatibility of luminal surfaces of cardiovascular prostheses. This review looks at the progress in *in vitro* expansion of and differentiating strategies for auricular chondrocytes and compares the mechanical qualities of tissue-engineered cartilage from human auricular chondrocytes to those of native auricular cartilage. Finally, some of the most promising approaches for the *in vivo* application of auricular chondrocytes/cartilage will be briefly discussed.

Recent advances in tissue engineering have made the formidable task of cartilage regeneration and its therapeutic use plausible. Different techniques are used to construct viable cartilage from chondrocytes, the cells that naturally form cartilage. Each of the 3 classes of cartilage – hyaline, elastic and fibrocartilage – has a distinct structure and biochemical nature, depending on its site-specific function. The composition of the extracellular matrix determines the differences between the chondrocytes found in the different types of cartilage.

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Tissue-engineered cartilage has to closely resemble native cartilage in terms of its size (thin), elasticity, contour (3-dimensional), growth, and sustainability *in vivo*. Other issues to be addressed in the use of tissue-engineered cartilage include the problems of dedifferentiation and decreased extracellular matrix and collagen deposition of matured chondrocytes (1-3). To overcome these limitations, investigators have been studying the use of growth factors, scaffolds, and other methods to improve tissue-engineered cartilage.

Biology of Chondrocytes

Chondrocytes, the resident cells of cartilage, lie isolated within an extracellular matrix in cavities called lacunae that are not vascularized or innervated. Because of its avascularity, cartilage is nourished by a superficial layer, the perichondrium (except for fibrous cartilage). Consequently, the chondrocyte is unique in its ability to exist in a low oxygen tension environment under normal and pathologic conditions. Partly as a result of these properties, cartilage has a low potential for repair, which in the case of articular cartilage, predisposes the tissue to degenerative conditions, such as arthritis (4). Chondrocytes undergo cell cycle arrest after skeletal maturity, except during conditions such as fractures or inflammation (5).

Several types of chondrocytes are found in the body, with the main difference between the chondrocytes being the surrounding matrix. Elastic cartilage comprises mainly elastin fibers along with type II collagen fibers and ground substance, which consists of interstitial fluid, proteoglycans, proteins, and carbohydrates that fill spaces in and around the fibers (6, 7). Aside from the components, elastic cartilage differs from hyaline cartilage in the spatial arrangement of the chondrocytes and in the amount of fibers. Found in the outer portion of the ear and in the epiglottis, elastic cartilage

contains more fibers and is more compact than hyaline cartilage. Elastic cartilage provides flexibility and support but is able to maintain its shape. Like hyaline cartilage, elastic cartilage is surrounded by a perichondrium.

Harvest of Auricular Chondrocytes

Auricular chondrocytes, found in the elastic cartilage of the ear, are easily accessible with minimally invasive methods (Figure 1) that significantly reduce scarring and morbidity (8). Harvesting methods have been studied in swine; open cartilage harvesting techniques involving suture closure were compared with a closed stitchless cartilage harvest, using a 12-gauge core biopsy needle. The closed technique required significantly less surgical time and no sutures. In addition, sample weights were significantly greater for the closed techniques. Furthermore, the incidence of hematoma, long-term stitch abscess, and scarring were lower with the minimally invasive closed technique. Finally, cell culture data showed similar cell growth characteristics for both techniques (9).

Harvesting of auricular cartilage has been examined in human studies. In one study, about 1 cm of auricular cartilage was collected from the auricular concha. The cells were grown in autologous serum to accelerate cell proliferation and produced a desired gel-form mass. Good to excellent cosmetic results were achieved in all patients (10).

Human auricular cartilage has been obtained in a prospective, *ex vivo* experimental model. To allow for injection through an 18-gauge needle into the desired tissue, the auricular cartilage was drilled down with a 5-mm otologic cutting bur, which allowed free passage. Cytologic examination of drilled septal cartilage showed good uniformity of cartilage pieces and occupation of 10% of lacunae by chondrocytes. In this study, other techniques tested (knife, morselizer and cartilage crusher) did not yield injectable cartilage slurries (11).

In addition to harvesting techniques, harvested chondrocytes must be adequately maintained for successful results. This issue may be especially important when chondrocytes are harvested from aged donors. Results from *in vitro* experiments on articular chondrocytes have indicated that proliferation and chondrogenic commitment are impaired in cultured chondrocytes from aged donors (12). Little is known about the effect of a patient's age on plasticity and proliferation capacity of auricular chondrocytes. However, chondrocytes produce less collagen, elastin and extracellular matrix *in vitro* after several passages (1, 2).

Expansion Methods of Auricular Chondrocytes/Cartilage

The number of chondrocytes for transplantation can be increased by repeated passaging of the cells in culture (Figure 2). Passaged chondrocytes are safe for implantation in generating tissue-engineered constructs; passaging does

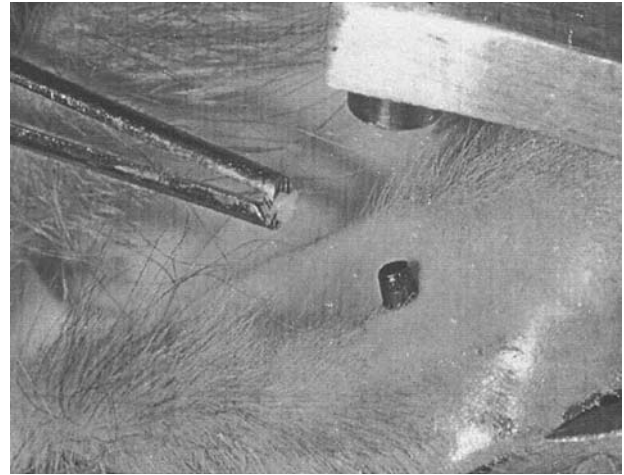


Figure 1. A 2-mm biopsy sample of auricular cartilage harvested with the use of a hole puncher from the ear of a calf. Note the minimal injury at the site of harvest (80).

not affect the normal diploid state of the cells (13). Supernatant from autologous platelets, with its multiple growth factors, enhanced chondrocyte and matrix synthesis of auricular chondrocytes (14). Repeated recycling of the culture medium may increase the number of chondrocytes *in vitro* (15). However, the quality of neocartilage has been shown to decrease from the third passage on (16). In addition, chondrocyte dedifferentiation is a problem during *in vitro* proliferation in monolayer culture. Dedifferentiated chondrocytes are characterized by a fibroblast-like phenotype; thus, repeated passage in monolayer reduces the efficacy of cartilage repair systems.

In a recent study comparing 3 types of sera for *in vitro* chondrocyte expansion, chondrocyte numbers increased 1000-fold at the third passage with whole blood-derived serum (WBS) and platelet-containing plasma-derived serum (PCS) and at the fourth passage with plasma-derived serum (PDS). Levels of epidermal growth factor, vascular endothelial growth factor, and platelet-derived growth factor were highest in WBS, followed by PCS and PDS. No significant differences were observed in histology or cartilaginous matrix measurements among the study groups (17). Alternatively, auricular chondrocytes obtained from children, were easily expanded in a combination of 2 standard culture media (Ham's F-12 and Dulbecco's Modified Eagle Medium) mixed with human fibrin (18).

Autologous auricular chondrocytes from swine maintained normal chondrocyte morphology when expanded *in vitro* with F-12 media supplemented with 10% autologous serum or growth factors. The chondrocyte yield was higher in F-12 media supplemented with growth factors than in F-12 media with autologous serum. These results suggest that both

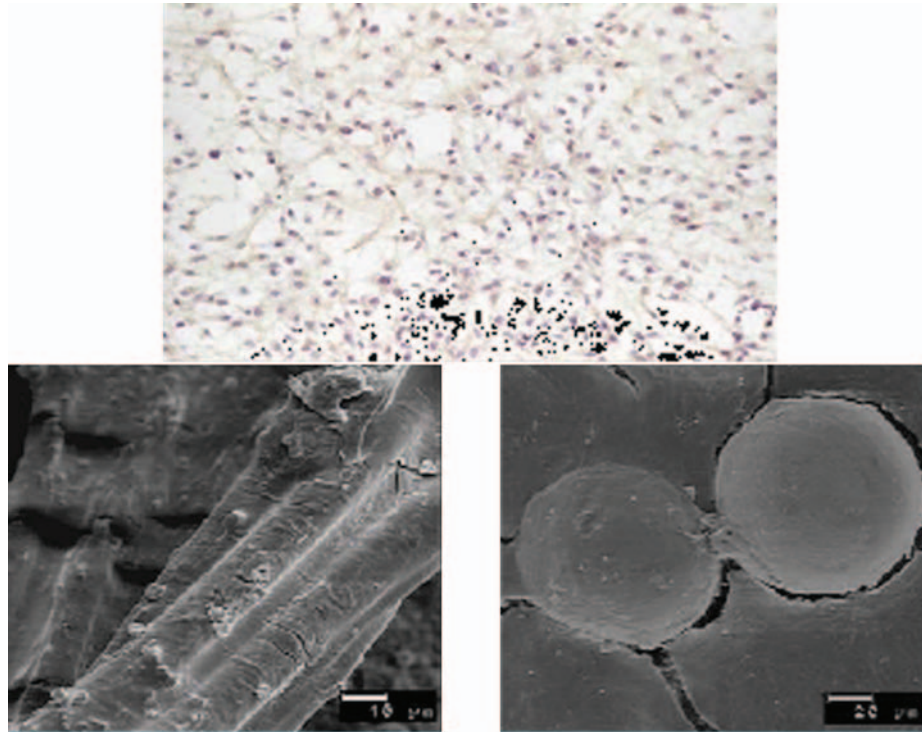


Figure 2. Immunocytochemistry of passed chondrocytes in a cell culture medium supplemented with vitamin C for improved collagen synthesis (top); SEM image of the biomaterial surface of a left ventricular assist device after 7 days of implantation showing an extensive amount of extracellular matrix and an intact, well-incorporated cellular coating on the textured polyurethane (bottom left) and sintered titanium (bottom right) surfaces (81).

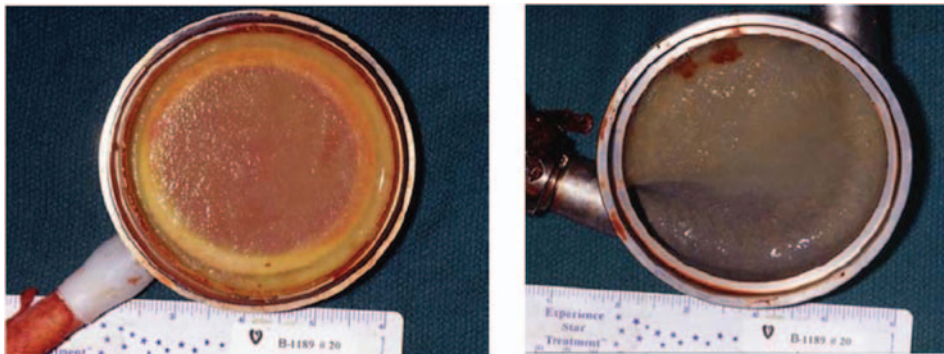


Figure 3. Gross appearance of a textured polyurethane (left) and sintered titanium (right) surfaces of left ventricular assist device that had been seeded with auricular chondrocytes and implanted in a calf for 7 days (8).

autologous serum and serum-free medium might be used to expand the number of chondrocytes, thus avoiding the potential need for a bovine serum supplement (19).

Wu *et al.* investigated the feasibility of platelet-rich plasma (PRP) as a carrier to deliver rabbit auricular chondrocytes and regenerate cartilage tissues *via* injection. Harvested chondrocytes were expanded *in vitro* and then mixed with PRP solution to generate chondrocytes/PRP

composites. Additional bovine thrombin was used for cross-linking, and then the composites were injected subcutaneously into the dorsal tissue of 4 cell donor animals. PRP alone was injected into 4 rabbits as controls. After 1 month, cartilage-like tissues were seen in the chondrocyte/PRP rabbit group as verified by magnetic resonance images and histologic analysis, whereas no tissue was found in the control (PRP only) group (20).

Several growth factors have been studied for their ability to stimulate proliferation of auricular chondrocytes. Transforming growth factor β (TGF- β) and basic fibroblast growth factor (bFGF) have consistently shown increased *in vitro* proliferation and extracellular matrix production (21-25). Supplementing bFGF with insulin or insulin-like growth factor 1 (IGF-I) synergistically increases proliferation. With repeated passaging, this combination increased cell numbers more than 10^4 -fold within 8 weeks (26). Elastic cartilage-derived cells cultured 2-dimensionally with bFGF and corticosteroids produced gel-type masses that became mature cartilage when injected into a subcutaneous pocket. In a study of the mechanisms of chondrogenesis, human elastic cartilage-derived cells from auricular cartilage, hyaline cartilage-derived cells from articular cartilage and mesenchymal stem cells from synovium were cultured in 3 media: "redifferentiation medium" containing bFGF and dexamethasone; "chondrogenic medium" containing bone morphogenetic protein-2, TGF- β 3 and dexamethasone specific for *in vitro* chondrogenesis of mesenchymal stem cells; and control medium. The elastic cartilage-derived cells cultured in redifferentiation medium produced a gelatinous matrix positive for glycosaminoglycans. During culture, the amount of chondroitin 4-sulfate, chondroitin 6-sulfate and hyaluronan increased. However, the expression of RNA for most chondrogenic genes did not increase. Only the injection of elastic cartilage-derived cells cultured with redifferentiation medium into the subcutaneous space of the nude mouse resulted in the formation of cartilage tissue (27).

In another successful combined approach, a cytokine-rich autologous serum (CRAS) system was developed. Canine auricular chondrocytes were cultured in medium supplemented with either fetal bovine serum (FBS) or autologous canine serum, alone or supplemented with bFGF. Cell proliferative capacity was higher in the CRAS cultures than in those cultured in FBS, and aggrecan and type II collagen expression was greater in the bFGF-supplemented CRAS group. The chondrocytes were subsequently seeded onto an ear-shaped biodegradable polymer and cultured in a bioflow reactor for 1 week, using the 3 different culture media indicated above. Then, they were implanted into nude mice. The best outcome (cartilage gene expression and morphologic properties) was seen with tissue-engineered constructs precultured in the bFGF-supplemented CRAS media (28).

Simulating a natural microenvironment – the use of scaffolds and bioreactors. Although investigators have demonstrated that neocartilage can be constituted in a predetermined shape and in complex 3-dimensional structures such as a human ear by using cell transplantation on polymer constructs, many problems are unsolved. Crucial issues for auricular tissue engineering include optimal cell culture environment, choice of polymers, behavior of chondrocytes, study of cell-polymer constructs in an acceptable animal model and long-

term structural integrity. Despite continuing obstacles in the field of auricular tissue engineering, there have been promising reports of different biodegradable biomaterial trials and the longest *in vivo* results of 10 months have been documented (29).

Various hydrogel materials originating from animals, plants or synthetic peptides have been tested as suitable scaffolds for cartilage tissue engineering. Human auricular chondrocytes were embedded in atelopeptide collagen, alginate or PuraMatrix. Chondrocytes proliferated well in the atelopeptide collagen but not in the other materials. A high cell-density culture within each hydrogel enhanced the expression of collagen type II mRNA. Additional stimulation with insulin and BMP-2 further significantly increased collagen type II and glycosaminoglycan accumulation within all hydrogels. Chondrocytes in the atelopeptide collagen expressed high levels of β 1 integrin, seemingly promoting cell-matrix signaling. N-cadherin expression was inhibited in the alginate, implying that a decrease in cell-to-cell contact may maintain chondrocyte activity. Considering the biologic effects and clinical availability, atelopeptide collagen may be accessible for clinical use. Because the use of synthetic peptides is not associated with the risk of disease transmission and immunoreactivity, improvement in their gelling ability would provide a more useful hydrogel for ideal cartilage (30).

Isogai *et al.* compared 4 types of bovine chondrocytes – nasoseptal, articular, costal and auricular – for tissue-engineered cartilage modeling. The chondrocytes were seeded onto biodegradable poly (L-lactide-epsilon-caprolactone) disc-shaped scaffolds. Cell-copolymer constructs were cultured and subsequently implanted in the subcutaneous space of athymic mice for up to 20 weeks. Levels of type II collagen and aggrecan gene expression were highest in costal chondrocytes, followed by nasoseptal, articular and auricular cells. Retrieval of 20-week discs from mice showed changes in construct dimensions with different chondrocytes. Greatest disc diameter was found for scaffolds seeded with auricular chondrocytes, followed by those with costal, nasoseptal and articular cells. Greatest disc thickness was measured for scaffolds containing costal chondrocytes, followed by those with nasoseptal, auricular and articular cells. Only auricular scaffolds developed elastic fibers after 20 weeks of implantation (31).

A scaffold composed of gelatin, chitosan and hyaluronan has been studied as material to mimic the composition of natural cartilage matrix for cartilage tissue engineering. Additional heparin and bFGF were incorporated into the scaffold. Rabbit auricular chondrocytes were seeded onto the ternary complex scaffold. No significant differences in glycosaminoglycan secretion were detected among the different scaffold groups; however, chondrocytes seeded in the scaffold-heparin-bFGF showed significant higher viability than those grown on the control (without heparin and bFGF) scaffold (32).

Human auricular chondrocytes cultured in 3-dimensional gel scaffolds expressed less collagen type I and had greater preservation of collagen type II than those cultured in monolayers. Moreover, scaffold-grown chondrocytes had favorable mechanical properties (33). These findings might be explained by the similarity of microenvironments; the cell-to-matrix adhesion or cell-to-cell contacts may closely resemble native cartilage (33, 34).

Auricular chondrocytes and articular cartilage can differ in cell behavior, growth and extracellular matrix production, which can affect neocartilage properties in tissue engineering approaches. Differentiation and proliferation are also affected by the surrounding microenvironment, including soluble factors, biomaterials and mechanical loading. When encapsulated in radically polymerized hyaluronic acid hydrogels, auricular chondrocyte constructs exhibited construct growth and neocartilage formation with an increase in tissue stiffness/consistency aggregate modulus and accumulation of extracellular matrix with culture. Articular chondrocyte constructs retained their construct size and translucency with a minimal increase in the compressive modulus. These data indicate that the specific cell source, cell/material interactions and loading environment are important in the final properties of tissue-engineered products (35).

Spongostan, a gelatinous haemostatic sponge used in surgery, was tested as a scaffold for chondrocytes developed from rabbit auricular cartilage. Foreign body giant cells often develop at the site of biomaterial implantation and may degrade spongostan. The results indicated that spongostan fulfilled its function as a cell scaffold; it did not induce inflammation but caused a local foreign body reaction resulting in its degradation (36).

Bioreactors may be used to deliver tissue-engineered cartilage. Physical stimulation affects chondrocyte function; therefore, bioreactors have been designed *in vitro* to transmit mechanical forces such as shear, hydrostatic pressure, compression and combinations of forces (37). Studies have demonstrated the pivotal role of robust 3-dimensional biomimetic microenvironments and indicate the potential of interactions between different cell types, such as mesenchymal stem cells and native chondrocytes, to create engineered cartilage (38). Pulsed ultrasound promoted cell proliferation and matrix deposition in low-density 2- and 3-dimensional chondrocyte cultures; however, the beneficial effects of ultrasound on neocartilage formation did not last as long as the beneficial effects of bioreactors (39).

Nanofibrous scaffolds, which have served as an extracellular matrix substitute, may be useful in cartilage tissue engineering. A key challenge in using nanofibrous scaffolds for tissue engineering is that the small pore size limits the infiltration of cells, which may result in uneven cell distribution throughout the scaffold. Li *et al.* seeded chondrocytes onto biodegradable nanofibrous scaffolds, and

the constructs were incubated with different growth factors. Constructs cultured in medium containing a combination of IGF-1 and TGF- β 1 expressed the highest mRNA levels of collagen type II and aggrecan. Radiolabeling analyses confirmed the effect on collagen and sulfated-glycosaminoglycan (sGAG) production. Histologic studies showed typical cartilage morphology throughout the entire structure of the constructs. When cultured using a rotary wall vessel bioreactor, the constructs formed a smooth, glossy cartilage-like tissue, whereas constructs maintained in a static environment formed a rough surface tissue. Bioreactor-grown cartilage constructs produced more total collagen and sGAG, resulting in greater net gain in tissue weight and improved mechanical properties. Together, these results indicate the applicability of nanofibrous scaffolds, combined with efficient cell loading and bioreactor technology, for cell-based cartilage tissue engineering (40).

Tissue-engineered cartilage vs. native cartilage. Bioengineering of cultured cartilage shows promise for clinical use (41). However, tissue-engineered cartilage with definite histologic and mechanical properties of native cartilage has not been produced. The mechanical and histologic properties of tissue-engineered derived from human auricular chondrocytes have been compared with those of native human auricular cartilage in athymic mice. After implanting the templates in the mice, the authors reported similar mechanical qualities in human chondrocyte-derived tissue-engineered cartilage and native auricular cartilage. Although histologic differences were noted, their results suggested that the engineered cartilage had sufficient strength and durability for clinical use (42).

Native and engineered cartilage tend to be brittle and fracture easily without a perichondrium. Expanded polytetrafluoroethylene (ePTFE) membrane has been used as a pseudoperichondrium to improve the flexibility of tissue-engineered cartilage. Swine auricular chondrocytes were suspended in fibrin glue, and the chondrocyte-fibrin glue composites were bound to ePTFE membranes and implanted into nude mice. Failure testing showed that specimens with the ePTFE membranes on both sides (cartilage core) returned to their original shape without fracturing even after rigorous torsion. Histologic analysis demonstrated that transplanted chondrocytes penetrated into the microporous structure of ePTFE and bonded to it (43).

In contrast, findings from a study on the feasibility and complications of transplanting sculpted autogenous tissue-engineered cartilage do not support their clinical use. In this study, all sculpted engineered cartilage specimens based on a synthetic scaffold of a polyglycolic acid and poly-L-lactic acid polymer lost their original 3-dimensional morphologic features. In addition, mass and chondrocyte viability were reduced in the engineered specimens 8 weeks after transplantation. These results were attributed to a reaction to

the polymer. Thus, morphologic features, osteogenic progression and reactions to foreign materials must be controlled for clinical applicability (44).

Alginate beads have been used to carry chondrocytes for subcutaneous cartilage formation in an autologous sheep model. The implanted constructs showed cartilage formation and retained a 3-dimensional shape. The proteoglycan and collagen contents of the constructs increased over time to up to 80% of the values for native tissue. The equilibrium modulus and the hydraulic permeability were comparable to those of native sheep auricular cartilage (45).

Autologous chondrocytes from rabbits were mixed with fibrin glue, and the mixture was injected along the nasal dorsa with various glue concentrations. After 8 weeks, the samples were explanted and compared with elastin and hyaline cartilage controls. In one-third of the rabbits, cartilage was formed along the injection sites. Higher thrombin concentrations positively correlated with successful creation of injectable cartilage. When hematoxylin-eosin and safranin O staining patterns were compared between rabbits treated with the fibrin glue mixture and normal auricular control cartilage, no differences were seen. In addition the presence of elastin fibers could be verified in the experimental group by Verhoeff staining. No foreign body reaction was observed (46).

Swine auricular chondrocytes were photoencapsulated either immediately after isolation (passage [p]=0) or after expansion (p=1 and p=2) in a hyaluronic acid hydrogel. These constructs were implanted subcutaneously in the dorsum of nude mice. After 12 weeks, the compressive equilibrium moduli of the p=0 and p=1 constructs were greater than those in the p=2 constructs and the control hyaluronic acid gel alone and were comparable to auricular cartilage. Histologic staining showed intense, uniform staining for aggrecan, as well as greater type II collagen versus type I collagen staining in all constructs. This study implies that neocartilage with mechanical and biochemical characteristics comparable to natural cartilage can be generated using this approach. However, prolonged *in vitro* expansion before implantation may compromise tissue properties (16).

Repair and Reconstruction

Ear reconstruction. Correcting microtia is challenging. A comparative research study investigated the potential of microtia cartilage chondrocytes with normal auricular chondrocytes as a source of tissue-engineered cartilage. Cartilage specimens from 12 pediatric patients (6 normal auricular specimens and 6 auricular specimens with microtia) were obtained and expanded *in vitro*. Each type of cell was implanted in nude mice to generate tissue-engineered cartilage. Both types of chondrocytes generated normal elastic cartilage upon histologic examination. Thus, microtia

cartilage may be an important additional donor source for the generation of a human tissue-engineered auricle (47).

In a study in rats, ear-shaped silicone molds were placed over transposed vessels in an abdominal wound pocket. In the presence of fibrin glue and the external mold, a vascular network formed that provided blood supply to auricular chondrocytes introduced into the molded capsule. Excellent shape was maintained in 4 of 6 cases with 2 of 6 having only minor superior pole deformation. In addition, all constructs were reliably transferred as free flaps (48).

Sanz *et al.* constructed matrices consisting of autologous immobilized auricular chondrocytes from rabbits and a collagen gel scaffold. Biopsy specimens of elastic cartilage were digested with collagenase II, and isolated chondrocytes were expanded in culture medium. Chondrocytes were immobilized into bovine collagen lattices and implanted, replacing pieces of removed native cartilage. New cartilage formed from implanted lattices with chondrocytes 5 weeks after implantation. Gross analysis of the ears showed that native and new cartilage were similar in appearance, consistency, texture and histologic composition. No signs of inflammation attributable to the implants were found in the control or the chondrocyte lattices (49).

Nasal reconstruction. Graft selection is a problem in nasal reconstruction in which the use of autologous cartilage provides the best resistance to infection and the lowest degree of resorption. As the nasal septum is often absent or insufficient in patients undergoing nasal reconstruction, the auricular concha offers a valid alternative (50). As the repair of a craniofacial or nose deformity requires a large volume of reconstructive material, augmentation of the facial form to the defect shape is difficult.

Auricular cartilage was collected from the auricular concha of patients age 9 to 63 years and expanded *in vitro* until it resembled a gel-form mass. This mass, together with autologous serum, was grafted (injected) on the periosteum and into the subcutaneous pocket. The lesion stabilized within 2 to 3 weeks. In all patients, aesthetic results were achieved and remained for periods ranging from 3 to 34 months. No case of absorption of cultured chondrocytes was seen. Biopsy specimens showed that the newly formed tissues were elastic cartilage derived from the original tissue (10).

Gane *et al.* used rolled autogenous conchal cartilage for dorsal augmentation of the minimally to moderately saddled nose. The use of an endonasal or external rhinoplasty approach achieved greater dorsal height than the more common layering techniques (51). To reconstruct severely retracted alar margins, Vural *et al.* used a turn-in flap brought from the nasal skin adjacent to the alar defect for inner lining, an auricular cartilage graft for alar margin support, and a 2-stage nasolabial flap for skin coverage (52). Auricular chondrocutaneous grafting may be used to

reconstruct alar defects because it matches the color, texture and contour of nasal tissue. However, the size of a composite graft that can be transferred is limited by the lack of blood supply to the area (53, 54).

The vascularized preauricular and helical rim flap is based on the superficial temporal vessels. Depending on the nature and size of the nasal defect, the *crus helicus*, helical rim, preauricular skin, superficial temporal fascia, and temporal bone can be harvested. In 61 of 63 (97%) patients, full-thickness nasal defects were successfully repaired, and a satisfactory outcome achieved in most cases (53).

The perichondrial cutaneous graft offers a reliable and dimensionally stable 1-stage reconstruction. The donor site is closed with the postauricular flip-flap. In a series of 41 consecutive patients, good or excellent aesthetic reconstructions were obtained in 39, whereas the graft failed in 1 patient, and 2 small postauricular wound dehiscences occurred (55).

Septal rhinoplasty is challenging. In a case series, Ribeiro and da Silva demonstrated an approach for closing septal perforations ranging from 1.0 to 3.5 cm in diameter. To correct the septal perforation and preserve vascularization of the columella and anterior septum, they used bilateral intranasal submucoperichondrial and submucoperiosteal advancement flaps with a sandwich graft interposition consisting of auricular or septal cartilage and 2 layers of deep temporoparietal fascia. The perforation was successfully closed in 257 of 258 patients (56).

Eyelid fornix reconstruction. Various methods have been reported for reconstructing the lower eyelid, but creating a deep fornix and a supportive eyelid for housing an artificial eye is challenging. In treating patients with anophthalmic orbits, achieving a long-lasting natural appearance and comfortable retention of eye prostheses is essential. Smith and Malet evaluated the surgical outcome of auricular cartilage grafts to correct inferior fornix retraction and eyelid malposition in 54 anophthalmic patients. Patients (age range, 5-86 years) presented with moderate to severe fornix retraction. The mean length of postoperative follow-up was 19.7 months. Successful correction was achieved in 92.6% of patients in the follow-up period; conjunctival fornix retraction recurred in 4 patients (57). Hashikawa *et al.* treated 16 patients with acquired anophthalmic orbit for lower lid retraction with an auricular cartilage graft. The aesthetic outcome was assessed as satisfactory in most patients and poor in only 4 patients. These 4 patients had undergone prior total maxillectomy with orbital exenteration or had eyelid defects because of previous cancer surgery (58). In a recent case report, a new prefabricated flap was developed using auricular cartilage and the lateral femoral circumflex vessels as vascular pedicles. The prefabricated flap was used in a 64-year-old man who had total lower eyelid loss after an extended maxillectomy for a tumor. This technique may improve

applications for use in patients with limited recipient vessels without major sacrifice or deformity in the donor area (59).

Laryngotracheal reconstruction. In a study of cartilage samples generated from auricular, articular and nasal chondrocytes, only auricular samples had the mechanical integrity and stiffness necessary to complete biomechanical testing. Furthermore, auricular samples had more consistent histologic features of the desired cartilage than did the other chondrocytes (60). Auricular chondrocytes may be useful in the surgical repair of laryngotracheal abnormalities or disabilities. *In vivo* studies revealed that autologous auricular cartilage implants provide excellent integration without any signs of inflammation or cartilage degradation. In contrast, tissue-engineered grafts and empty scaffolds showed marked signs of an unspecific foreign body reaction, which led to a complete degradation of the neocartilage, whether implanted para- or intralaryngeally (61). To overcome the issue of foreign body reaction, auricular chondrocytes were used in a rabbit study to engineer scaffold-free cartilage sheets. Autologous neotracheal constructs consisting of cartilage, a muscle flap and a silicone stent were implanted in the abdomen of 6 New Zealand white rabbits to fabricate a vascularized neotrachea *in vivo*. In 2 of the 6 rabbits, a full thickness skin graft was used to create an epithelial lining. After 6 weeks all neotracheal constructs were healthy with well-vascularized and integrated layers. The implanted engineered cartilage underwent a remodeling process, forming a solid tracheal framework. Constructs harvested after 10 weeks had significantly better mechanical properties than after 6 weeks and were comparable with the rabbit's native trachea (62).

Tension is the limiting factor in long tracheal resection with end-to-end anastomosis. Complications such as dehiscence and restenosis correlate with the degree of anastomotic tension. Zaugg *et al.* demonstrated that gluing patches of sheep auricular cartilage with albumin-glutaraldehyde tissue adhesive (BioGlue) craniocaudally along a tracheal anastomosis significantly strengthened the anastomosis and helped decrease tension on the suture line (63).

Joint reconstruction. Several chondrocyte sources have been evaluated for the ability to produce new cartilaginous matrix *in vivo* and form functional bonds with native cartilage disks of articular cartilage. Articular, auricular or costal chondrocytes were harvested from swine, suspended in fibrin glue (experimental), placed between disks of articular cartilage and implanted subcutaneously into nude mice for 6 and 12 weeks. New matrix, consisting mostly of neocartilage integrating with the cartilage disks, formed in all experimental mice, whereas control mice (no chondrocytes) developed fibrous tissue without evidence of neocartilage. Furthermore, tensile strength was higher in all experimental samples than in controls (64).

Lumbar intervertebral discs (IVD) were studied in 16 New Zealand white rabbits. In the experimental group the nucleus pulposus from 6 IVD was evacuated and replaced with tissue-engineered autologous chondrocytes from auricular cartilage. Autologous cartilage implants were well tolerated by the host for up to 6 months *in vivo*. Hyaline-like but not elastic cartilage was seen in the place of the nucleus pulposus (65).

Auricular, articular and costal chondrocytes have been seeded onto Vicryl mesh to repair an artificial lesion in the avascular zone of the meniscus. In all experimental samples, lesion closure was observed, and gross mechanical testing showed bonding of the lesion. Histologic analysis showed formation of new tissue in experimental but not control samples. Surprisingly, auricular chondrocytes achieved similar results as articular chondrocytes (66).

Auricular cartilage grafts were successfully used in reconstruction of ankylosed temporomandibular joints (TMJ) in sheep. TMJ ankylosis was induced in the right joints of 5 sheep; the left joints were used as controls. The ankylosed TMJ was released by gap arthroplasty with an interposed auricular cartilage graft at 3 months, and 12 weeks later the sheep were euthanized. Maximal mouth opening was measured pre- and postoperatively. Despite radiographically irregular surfaces of the temporal bone and ramus stumps radiolucent gaps were formed between them. Histologic study showed the auricular cartilage graft was alive and well attached to the mandibular ramus stump. In all operated joints, joint space was seen between the grafted cartilage and temporal bone, and the space was filled with fibrous connective tissue oriented parallel to the temporal surface (67). Jain *et al.* have described how auricular cartilage implants were used as a common alternative to gap arthroplasty in a study of 44 patients with TMJ ankylosis (68).

Intrinsic sphincter deficiency and vesicoureteral reflux. Intrinsic sphincter deficiency (ISD), caused by weakening of the sphincter muscle, results in incontinence and vesicoureteral reflux. Reflux occurs when urine flows back up the ureter and, in severe cases, into the kidney. Usually seen in young children, reflux can cause infection and can damage the kidneys. In a study of 29 children with reflux, autologous auricular chondrocytes were cultured, prepared as a gel in calcium alginate, and then injected using endoscopic guidance. The chondrocyte mixtures, placed into critical areas, significantly improved the vesicoureteral reflux (69). Because of the possibility of relapse, changes in the formulation of the material have been made to enhance implant reliability and increase long-term success. Treatment of ISD with endoscopic injection of chondrocytes distal to the bladder neck has improved lifestyle and decreased incontinence. In 32 patients who received a single injection of chondrocytes, 50% (16/32) were dry 12 months after 1

injection. In 26 of 32 patients who were dry or improved at 3 months after injection, the effect was maintained at the 12-month visit (70).

Areola and nipple reconstruction. A mastectomy is often performed in the treatment of breast cancer. The desired goal for reconstructive surgery in a patient who has undergone a mastectomy is a natural-looking and -feeling nipple-areola complex. Plastic surgery should result in a reconstructed breast that is similar in symmetry, color, shape and size to the other breast.

Cao *et al.* compared the suitability of 3 polymers for generating tissue-engineered elastic pig cartilage. Chondrocytes were isolated onto fiber-based poly(glycolic acid) (PGA) scaffolds or suspended in calcium alginate or pluronic F127 gel. Chondrocyte-polymer constructs were either implanted (PGA) or injected (calcium alginate and pluronic) as autologous implants subcutaneously into the pigs from which the cells had been isolated. After 6 weeks *in vivo* all explants demonstrated varying degrees of cartilage formation. Histologic study showed the use of PGA or calcium alginate yielded tissue formed of fibrocartilage with thick bundles of collagen dispersed in the tissue. The use of pluronics as a scaffold resulted in histologic features resembling those of native elastic cartilage, showing a more organized arrangement of the cells (71).

In a second study, Cao *et al.* demonstrated an effective approach to creating autologous tissue-engineered cartilage in the shape of a human nipple by injecting a reverse thermosensitive polymer seeded with autologous chondrocytes in immunocompetent pigs. Polyethylene oxide and polypropylene oxide (Pluronic F-127), which exists as a liquid below 4°C and polymerizes to a thick gel at body temperature, was used as a vehicle for autologous chondrocytes. The constructs were injected on the ventral surface of the pigs and a circumferential subdermal suture was used to support the contour of the implant and assist in its projection in the form of a human nipple. After 10 weeks, visual inspection of the tattooed chondrocyte-Pluronic F-127 hydrogel implant sites revealed that they closely resembled a human nipple-areolar complex. Nodules were similar in size, shape, and texture to a human nipple at each injection site. Glistening opalescent tissue was surgically isolated from each implant site with the characteristic histologic signs of elastic cartilage (72).

In a different surgical approach for areola repair, rolled auricular cartilage was placed in the center of the bridge of the dermal base and was wrapped with bilobed dermal-fat flaps. Because of its composition, the graft maintained good circulation, and necrosis in the flap could be prevented. In addition, cartilage produces and sustains a good form of the feature without subcutaneous depression because the cartilage is supported by the bridge of the dermal base (73).

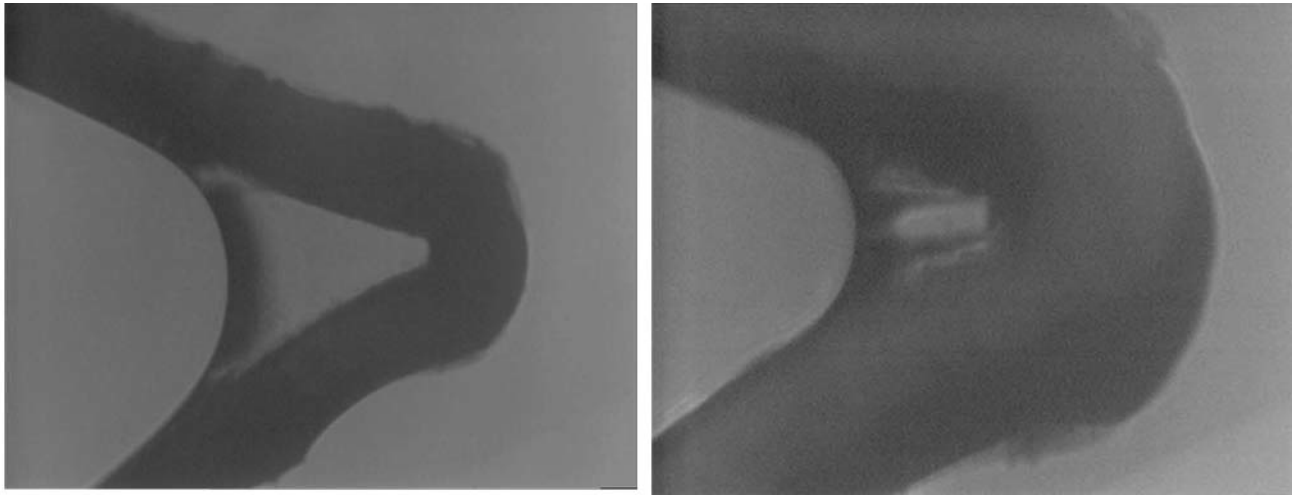


Figure 4. Monofilament nitinol stent before (left) and after (right) expansion. The stent was seeded with auricular chondrocytes, which were allowed to grow completely to cover the stent. Visual microscopy confirmed the chondrocyte coating of the stent remained intact even after expansion (magnification, $\times 1000$; wire filament width, $200\ \mu\text{m}$) (79, 81).

Cell lining of artificial surfaces. Cell lining helps increase the biocompatibility of cardiovascular prostheses. In early investigations the use of endothelial cells to line artificial surfaces showed short-term improvement in biocompatibility, but long-term outcome was unsatisfying (74, 75).

Alternatively, genetically engineered smooth muscle cells (SMCs) overexpressing endothelial nitric oxide synthase (NOS III) have been used to form a nonthrombogenic cellular lining for the Thermo Cardiosystems Heartmate left ventricular assist device (LVAD) (76, 77). Cumulative cell loss from cell-lined LVADs was less than 10% after 24 hours of flow. Subsequently, an LVAD containing a genetically engineered SMC lining was successfully implanted in a bovine model. Results from this 24-hour study indicated that the flow-conditioned cellular lining remained intact with no evidence of thromboembolization and only minimal changes in coagulation studies (78).

In a proof-of-principle study, auricular chondrocytes were used to line 4 LVADs. Histologic and immunochemical studies showed evidence of pure elastic cartilage and the presence of chondrocytes in the LVADs. The efficiency of seeding chondrocytes onto the luminal surfaces of the LVADs was nearly 100%, and cell loss was fairly low during preconditioning under flow conditions *in vitro* (Figure 3). More importantly, after 1 week *in vivo*, an intact, strongly adherent cellular lining was seen on the luminal surfaces of the implanted LVADs, and no adverse thrombogenic events occurred (8).

In a feasibility study to improve biocompatibility, a monofilament nitinol stent was seeded with auricular chondrocytes, which were allowed to grow until they completely covered the stent (Figure 4). At the end of day 4, the stent was expanded. Microscopic analysis showed that

the chondrocyte coating remained intact after expansion (79). The simple method of isolating and expanding auricular chondrocytes could be used to provide strongly adherent autologous cell linings for LVADs and other commonly used cardiovascular devices.

Conclusion

Surgical results with auricular cartilage have been aesthetically positive, with a low risk of complications or other adverse effects to the donor. Harvesting cartilage from the auricular concha is less traumatic than obtaining cartilage from rib or knee joints. Progress has been made in successfully obtaining and expanding auricular chondrocytes and in inducing chondrogenesis *in vitro*. Culture medium supplements such as bFGF and IGF-1 have increased chondrocyte proliferation. The use of biodegradable polymers/scaffolds has increased *in vitro* chondrocyte expansion and chondrogenesis. In addition, polymers/scaffolds can serve as a chondrocyte carrier for transplantation. The use of bioreactors further optimizes *in vitro* expansion rates for chondrocytes without a loss of their phenotype. A combination of supplements, scaffolds and bioreactors delivers tissue-engineered cartilage with morphological and immunohistochemical characteristics of native tissue. Nevertheless, challenges remain. Some studies suggest that foreign body reactions directed against the scaffold material may lead to disintegration of the scaffold/cell construct *in vivo*.

Tissue-engineered cartilage may be an alternative for autologous cartilage for repairing tissue defects. Although similar, the mechanical and biologic properties of tissue-

engineered cartilage do not yet match those of native auricular cartilage. To address this challenge, auricular chondrocytes are being used in combination with biomechanical carriers and various scaffolds. In addition, preshaped molds may broaden the use of engineered cartilage by improving chondrocyte survival and mechanical function. Auricular chondrocytes and auricular cartilage have been widely studied and have shown promise in experimental settings and in reconstructive surgery. Interestingly, auricular chondrocytes/auricular cartilage provided equal if not better mechanical properties than cartilage from other body sites in some study settings. Auricular cartilage has gained importance for lower eyelid and nose repair grafts, either directly or as part of a flap. In addition, auricular chondrocytes and cartilage have been used for laryngotracheal, joint and ear repair. Older studies have reported on the possible use of auricular chondrocytes/ cartilage for mammary/areola reconstruction and for cases of bladder sphincter insufficiency. Furthermore, auricular chondrocytes have formed intact layers on artificial surfaces of cardiac devices such as LVADs and coronary artery stents. These layers remained intact even under systemic flow conditions and may improve biocompatibility and longevity of implantable cardiac devices.

Auricular cartilage and chondrocytes are being used for a variety of applications in many different medical and surgical fields. The rapid advances in tissue engineering technologies and the pleiotropic biological properties of auricular chondrocytes make these cells a versatile option for future cell and tissue-based therapies.

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