Guided Cartilage Regeneration Using Resorbable Template

Bohdan Pomahac, MD, Baraa Zuhaili, MD, and Yusuf Kudsi, MD

Division of Plastic Surgery, Brigham and Women's Hospital, Harvard Medical School

Correspondence: bpomahac@partners.org

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Background: The reconstruction of a defect involving complex cartilaginous structures such as the ear and nose is a difficult problem. Cartilage donor sites are limited, and the shaping of an ear or nose is dependent upon the surgeon’s skills and experience. In this report, we propose to use resorbable plates that can be shaped to serve as a template for cartilage healing.

Methods: A shell composed of polylactic/polyglycolic acid copolymer sheet was molded into different shapes. Autologous ribs harvested from 2 New Zealand rabbits were slightly crushed and bent without breaking, and placed within the pre-shaped shell. The constructs were implanted into subcutaneous pockets in the flanks of the rabbits. After 8 weeks, the implanted cartilage constructs were taken out of the shell and analyzed by the gross macroscopic appearance for preservation of the shape and by histological means for analysis of cartilage viability.

Results: All of the explanted cartilage constructs retained the same pre-implanted shape and contour. Upon histological examination with hematoxylin/eosin staining, the constructs were composed of a continuous layer of viable chondrocytes.

Conclusions: Construction of complex cartilaginous structures is an operator-dependent, technically difficult problem. We propose to use a resorbable template for guiding the shape and healing of the desired cartilaginous construct. Preoperative scanning and precise 3-dimensional shaping of the template could achieve further improvement in the desired cartilaginous support of the reconstructed part. In this report, we document that cartilage enclosed in a resorbable template retains its shape and viability. We believe that a prefabricated shell may help simplify and standardize outcomes of ear or nose reconstruction.

The ear and nose are organs with specific and complex anatomical structures. Replacement or construction of either one requires multiple, staged operations. The clinical outcome is variable and highly dependent on the surgeon’s skill. It is well documented that extensive experience is mandatory and markedly improves the results. Autologous costal cartilage is the most commonly used source of cartilage for complete ear construction. Brent and Nagata’s surgical techniques involve 4 and 2 surgical stages respectively, and both require a “unique marrying of science and art.”

An effort towards more reliable and reproducible techniques with less donor site morbidity led to the utilization of irradiated homograft, xenograft, or alloplastic materials. The
most common problem unifying these techniques has been the risk of infection and poor integration. On the other hand, the methods of tissue engineering have been proposed to create a cartilaginous tissue starting from isolated chondrocytes seeded in vitro to scaffolds. Although exciting in theory, the results have been largely disappointing, and newly formed constructs rarely held their shape.

In an effort to develop a standard surgical technique for ear and nose reconstruction, we are studying the possibility of utilizing autologous costal cartilage to create a complex shape regardless of the surgeon’s experience. Thus, we examined the possibility of guiding the shape and healing of the cartilage with a resorbable template. In this study, we used LactoSorb as a template and studied the shape and viability of the constructed cartilage.

MATERIALS AND METHODS

All animal experiments were approved by the Harvard Standing Committee on Animals.

Harvesting costal cartilage

Two New Zealand white rabbits (4 weeks old, 1 kg) were anesthetized with a mixture of xylazine (5 mg/kg body weight) and ketamine (45 mg/kg body weight) injected intramuscularly into the hind limb thigh according to methods described previously. A sterile surgical field was created by shaving the designated area with an electric clipper and then preparing the area. The incision site was located over the medial part of the costosternal junction, and the cartilaginous parts of the rib were harvested. Great attention was paid not to penetrate the pleural cavity during dissection. After careful hemostasis with electric cautery, the incision was closed in a layered fashion.

Construct of the shell implant

For creation of the implant, we used the resorbable material LactoSorb. These polymers are rigid at room temperature but are moldable when warmed with hot air or water to 60°C. Each of the sheets was warmed up to the malleable state in a sterile hot water bath. Then each sheet was folded around the metal template to create an implant with a specific shape.

Creation of the cartilage-shell construct

Previously obtained cartilage was crushed slightly by applying direct force of a needle holder to soften the surface without breaking or disrupting the continuity of the cartilage as described previously. The first cartilage piece was molded into an S-shaped construct; the second cartilage piece was molded into a letter U-shaped construct. For control purposes, another construct with similar shape was created and left empty.
Implantation of the cartilage-shell construct

Rabbits’ flanks were prepared surgically as described above. A small subcutaneous pocket was created by blunt dissection on each side of the flank, with great attention paid to securing enough loose skin to accommodate the implant without jeopardizing the viability of the skin. The previously described cartilage-shell construct was implanted on one side, and an empty shell construct was implanted on the other side for control purposes. The incision was then closed in layers.

Explantation of the cartilage-shell construct

Eight weeks after implantation, the 4 constructs were removed from the 2 rabbits and the rabbits were euthanized.

HISTOLOGY

The retrieved constructs were cut open, and the tissue inside was immersed in formalin aldehyde solution and sent for histological study.

Tissue sections were stained with hematoxylin and eosin (H & E) for morphologic analysis.

RESULTS

Gross findings

Upon extracting from LactoSorb constructs, both the S-shaped and the U-shaped constructs were consistently rigid with preserved continuity. There were no visual breaks through either construct. The color of both constructs was light ivory (Fig 1). When examining the construct’s contour, both structures were identical to that of the original implant as shown in Figure 1.

Figure 1. Macroscopic appearance of the extracted S-shape construct (magnification bar = 3000 μm).
Examination of the control constructs revealed fragile fibrous-like tissue only (data not shown in figures).

Figure 2. Cross section of the extracted S-shape construct. No disruptions of the cartilaginous tissue were noted (hematoxylin/eosin staining, magnification: 10×, magnification bar = 3000 μm).

Figure 3. Cross section of the end of extracted S-shape construct with viable chondrocytes (hematoxylin/eosin staining, magnification: 40×, magnification bar = 750 μm).
Microscopic findings

Histological slides stained with hematoxylin/eosin showed that a continuous layer of chondrocytes was noted throughout the construct as shown in Figure 2. Within that continuous layer, an organized set of viable chondrocytes was embedded in an extracellular matrix as shown in Figure 3.

In an unpublished set of experiments, cartilages were cut into pieces and implanted subcutaneously. Interestingly, the cartilage did not heal and rather encapsulated in fibrous tissue as shown in Figure 4.

![Figure 4](image)

Figure 4. Microscopic appearance of cartilaginous pieces examined 8 weeks after subcutaneous implantation. Chondrocyte islands are encapsulated in fibrous tissue (hematoxylin/eosin staining, magnification: 100×, magnification bar = 500 μm).

DISCUSSION

More than 1 million patients undergo cartilage reconstruction-related procedure every year in the developed countries. Autologous cartilage is the preferred source eliminating the risk of infection transmission while maintaining its biocompatibility. Problems are related to donor site morbidity and limited availability. Cartilage can be also obtained from unrelated donor (homograft), bovine (xenograft), or replaced by alloplastic material. Irradiated homografts have been popular because of demonstrated safety, ease of use, and immediate availability that decrease the operating room time.
Alloplastic materials, while also readily available, carry the risk of infection and poor integration.8

Tissue engineering techniques seemed promising to provide almost unlimited supplies of cartilage by in vitro expansion from small amount of donor tissue. In theory, the isolated chondrocytes are expanded in vitro, seeded into resorbable matrix that provides an attachment network in the desired shape.9 Cellular proliferation then transforms this construct into a mature appearing cartilage. Yet, these attempts have not yielded reliable results because of fast chondrocyte in vitro de-differentiation,10 chondrocytes’ catabolic tendency resulting in loss of tissue engineered construct shape,11 and the inability to address the common shortage of vascularized overlying tissues.12

External silicone prosthesis can yield a realistically appearing outcome and, however, requires daily routine of cleaning, overnight removal, and in noncompliant patients, may result in pin infection.13

Prosthetic reconstruction using polyethylene implants wrapped in vascularized tissues are suitable for selected patients.8 A majority of ear and nose reconstruction is, however, performed by shaping the costal cartilage pieces with often frustrating results related to the surgeon’s skills.

The total ear construction for the treatment of microtia was first achieved in the early 1930s by Pierce and others.14,15 Since then, different formulas for ear reconstruction were based on variations on the same theme. Tanzer introduced the 4-stage standardized way of ear reconstruction.16 In the first stage, the lobular remnant is transposed to its anatomic position. In the second stage, costal cartilage, harvested from the sixth, seventh, and eighth ribs, is implanted beneath the mastoid skin. The sixth and seventh costal cartilages are used for the base and antihelix, and the eighth costal cartilage becomes the helical rim. In the third stage, the construct is elevated from the cranium by forward movement of postauricular skin and placement of a retroauricular, full-thickness skin graft. The concha and tragus are subsequently created with composite contralateral ear and skin/cartilage grafts. Tanzer later modified this sequence by combining the lobular transposition and placement of the cartilage framework into 1 stage.17

This technique was further developed by Brent et al.18,19 Brent’s technique consists of 3 or 4 stages. In the first stage, the pattern for the construct is made by cutting an x-ray film, copying the normal ear and harvesting the contralateral sixth, seventh, and eighth costal cartilages. Within the same stage, Brent fabricates the cartilage according to the landmarks of the opposite ear and places the resultant construct into a subcutaneous temporal pocket. Lobule transposition is performed in the second stage. The construct is elevated in the third stage to achieve projection of the helical rim, while tragus construction, conchal excavation, and symmetry adjustment are performed in the fourth stage.

Introduced later on in 1993, Nagata’s technique involves 2 stages.20 The first stage roughly corresponds to the first 3 stages in the Brent technique.21 In the first stage, the rib cartilage framework is placed in a subcutaneous pocket and the lobule is transposed. Six months after the first stage, the construct is elevated to the final position and a temporoparietal fascia flap is then used to cover the reconstructed auricle.

So far, autologous costal rib cartilages are considered the most accepted source for cartilaginous organs reconstruction14 with focus on staging of the surgical procedure. We perceive that shaping of the cartilage is a similarly cumbersome and difficult task and that standardization using modern techniques could simplify this problem.
Although not common in cartilaginous organ reconstruction procedures, resorbable plates are widely used in pediatric craniofacial surgery for internal fixation. These plates, similar in composition to resorbable sutures, have also been used for “internal splinting” of the cleft lip-nasal deformity following primary rhinoplasty. The material dissolves over 12–24 months by hydrolysis. It is biologically inert and hence causes only a minimal inflammatory reaction.

In this study, we used a poly-L-lactic/polyglycolic acid template, commercially available as LactoSorb, to maintain the desired cartilage shape. We showed that cartilage remains viable until the end of 8 weeks following implantation and that it maintains the shape of the template. In this technical report, we want to point out a possible new avenue that may simplify ear reconstruction. We postulate that constructing a complex cartilaginous structure may be accomplished more easily by computer scanning of the contralateral ear with subsequent mirror image translation into the final shape of the template. The harvested costal cartilage would then be used to fill the template, and within the same stage could be placed in the final position.

Surgeons have utilized the technique of cartilage crushing to increase the malleability of the cartilage without breaking. This largely subjective technique depends on the thickness and origin of the cartilage that changes the force that needs to be applied. Cakmak et al found through a scored crushing system that the viability of the cartilage is inversely related to the intensity of the crushing. In his report, however, he uses the terms slight, moderate, significant, and severe crushing that is difficult to apply among different species or source of cartilage. Description of crushing by the force applied to rabbit costal cartilage is limited by the nature of the tissue, that varies in thickness from one to the other end. We, therefore, feel that description of crushing is best achieved in conjunction with the preserved continuity of the cartilage.

Cartilage cutting or mincing does not lead to healing, but rather encapsulation in fibrous tissue (Fig 3). This emphasizes the importance of preventing breaks in cartilage.

A creative way to solve the problem of vascularity was presented by Neumeister, who managed to create a vascularized capsule around a cartilaginous construct by transposition of the femoral artery in rats.

In summary, we think that further molding of the plate guided by a digital image of the contralateral ear could lead to the preparation of a resorbable template that would be a precise mirror image of the opposite side. It is our hope that this report will contribute to a better standardization of the difficult reconstruction of the ear.

REFERENCES

1. Brent B. Auricular repair with autogenous rib cartilage grafts: two decades of experience with 600 cases. Plast Reconstr Surg. 1992;90(3):355–74; discussion 375–6.
2. Willers C, et al. Autologous chondrocyte implantation with collagen bioscaffold for the treatment of osteochondral defects in rabbits. Tissue Eng. 2005;11(7/8):1065–76.
3. Cakmak O, et al. Viability of crushed and diced cartilage grafts: a study in rabbits. Arch Facial Plast Surg. 2005;7(1):21–6.
4. Cakmak O, et al. Viability of cultured human nasal septum chondrocytes after crushing. Arch Facial Plast Surg. 2005;7(6):406–9.
5. Chang SC, et al. Tissue engineering of autologous cartilage for craniofacial reconstruction by injection molding. *Plast Reconstr Surg.* 2003;112(3):793–9; discussion 800–1.

6. Strauch B, Wallach SG. Reconstruction with irradiated homograft costal cartilage. *Plast Reconstr Surg.* 2003;111(7):2405–11; discussion 2412–3.

7. Adams WP, Jr, et al. The rate of warping in irradiated and nonirradiated homograft rib cartilage: a controlled comparison and clinical implications. *Plast Reconstr Surg.* 1999;103(1):265–70.

8. Cenzi R, et al. Clinical outcome of 285 Medpor grafts used for craniofacial reconstruction. *J Craniofac Surg.* 2005;16(4):526–30.

9. Cao Y, et al. Transplantation of chondrocytes utilizing a polymer-cell construct to produce tissue-engineered cartilage in the shape of a human ear. *Plast Reconstr Surg.* 1997;100(2):297–302; discussion 303–4.

10. Schulze-Tanzil G, et al. Loss of chondrogenic potential in dedifferentiated chondrocytes correlates with deficient Shc-Erk interaction and apoptosis. *Osteoarthritis Cartilage.* 2004;12(6):448–58.

11. Christophel JJ, Chang JS, Park SS. Transplanted tissue-engineered cartilage. *Arch Facial Plast Surg.* 2006;8(2):117–22.

12. Ciorba A, Martini A. Tissue engineering and cartilage regeneration for auricular reconstruction. *Int J Pediatr Otorhinolaryngol.* 2006;70(9):1507–15.

13. Gion GG. Surgical versus prosthetic reconstruction of microtia: the case for prosthetic reconstruction. *J Oral Maxillofac Surg.* 2006;64(11):1639–54.

14. Beahm EK, Walton RL. Auricular reconstruction for microtia: part I. Anatomy, embryology, and clinical evaluation. *Plast Reconstr Surg.* 2002;109(7):2473–82; quiz following 2482.

15. Walton RL, Beahm EK. Auricular reconstruction for microtia: Part II. Surgical techniques. *Plast Reconstr Surg.* 2002;110(1):234–49; quiz 250–1, 387.

16. Tanzer RC. Total reconstruction of the auricle. The evolution of a plan of treatment. *Plast Reconstr Surg.* 1971;47(6):523–33.

17. Tanzer RC. Microtia—A long-term follow-up of 44 reconstructed auricles. *Plast Reconstr Surg.* 1978;61(2):161–6.

18. Brent B. The correction of microtia with autogenous cartilage grafts: I. The classic deformity. *Plast Reconstr Surg.* 1980;66(1):1–12.

19. Brent B. The correction of microtia with autogenous cartilage grafts: II. Atypical and complex deformities. *Plast Reconstr Surg.* 1980;66(1):13–21.

20. Nagata S. A new method of total reconstruction of the auricle for microtia. *Plast Reconstr Surg.* 1993;92(2):187–201.

21. Kawanabe Y, Nagata S. A new method of costal cartilage harvest for total auricular reconstruction: part I. Avoidance and prevention of intraoperative and postoperative complications and problems. *Plast Reconstr Surg.* 2006;117(6):2011–8.

22. Wong GB, Burvin R, Mulliken JB. Resorbable internal splint: an adjunct to primary correction of unilateral cleft lip-nasal deformity. *Plast Reconstr Surg.* 2002;110(2):385–91.

23. Neumeister MW, Wu T, Chambers C. Vascularized tissue-engineered ears. *Plast Reconstr Surg.* 2006;117(1):116–22.