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Cartilage Regeneration in the Rabbit Nasal Septum

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Objective: **Rhinoplasty frequently includes harvesting of nasal septal cartilage. The objective of this prospective basic investigation is to determine whether cartilage can regenerate after submucosal resection (SMR) of the nasal septum in the rabbit. Neocartilage formation has not heretofore been described in this model.** ***Methods:*** **By lateral rhinotomy, SMR was performed on 17 rabbits followed by reapproximation of the perichondrium. After 7 months, septi were fixed, sectioned, and examined histologically. Findings were photographed and data tabulated according to location and extent.** ***Results:*** **Sites of matrix-secreting isogenous chondrocyte islands were identified between the perichondrial flaps of every animal, principally in the anterior inferior septum. The width of the islands averaged 190 μm, and the mean neocartilage height was found to be 840 μm. The newly formed cartilage consisted of chondrocytes within chondrons and was comparable in shape and structure to native septal cartilage.** ***Conclusions:*** **After SMR, rabbit cartilage tissue can regenerate and form matrix within the potential space created by surgery. The surrounding stem cell-rich perichondrium may be the site of origin for these chondrocytes. These findings suggest that after SMR of the human nasal septum, it may be possible for new cartilage tissue to develop provided the mucosa is well approximated. This biologic effect may be enhanced by insertion of cytokine-rich tissue scaffolds that exploit the native ability of septal perichondrium to regenerate and repair cartilage tissue.** ***Key Words:*** **Cartilage, nasal septum, submucosal resection (SMR), perichondrium, regeneration, rabbit, cytokine, scaffold.**

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INTRODUCTION

Rhinoplasty often requires the use of septal cartilage for grafting. Unfortunately, the amount of septal cartilage available for surgery is often inadequate, particularly in secondary rhinoplasty, in which a cartilaginous defect results from previous graft harvest or submucosal resection (SMR) of the quadrangular cartilage. Creating new cartilage within SMR defects would not only prevent the complications that occasionally result from the absence of septal cartilage such as septal perforation and saddle-nose deformities, but potentially provide graft material for use in future aesthetic and reconstructive procedures while avoiding the quantitative limitations, donor site morbidities, and differences in biochemical composition and biomechanical behavior that may accompany the use of current autologous sources such as costal or auricular cartilage.

Therefore, any insight into the natural history of facial/nasal cartilage synthesis after SMR is important, because carrier vehicles for cytokines or artificial scaffolds may be introduced into these defects to spur chondrogenesis. Chondrocytes derive from pluripotent mesenchymal stem cells.[1,2] Perichondrium, which surrounds cartilage tissue in the face and airway, serves as a reservoir for mesenchymal stem cells.[2] Migration of these stem cells into the healing wound, followed by differentiation, is regulated by the spatiotemporal biochemical milieu.[3] In young rabbits, growing nasal septal cartilage shows both cell proliferation and matrix production, leading to new cartilage formation. In contrast, healing in older rabbits tends more toward fibrosis without evidence of new hyaline cartilage formation.[4] In the current study, we investigate the long-term potential for cartilage regrowth after SMR of the nasal septum in the mature rabbit, because this is the first step toward developing technology to regenerate cartilage in SMR defect sites.

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MATERIALS AND METHODS

The experiment was carried out with the approval of the Institutional Animal Care and Use Committee at the University of California, Irvine.

Seventeen mature New Zealand white rabbits aged 9 to 12 months and ranging in weight from 3.5 kg to 4.5 kg underwent rhinotomy and partial submucosal resection of the cartilaginous nasal septum as described previously.[5] Briefly, after induction of general anesthesia, the snouts were shaved and prepped for surgery and then a 3.0-cm nasal dorsal incision from the level of the frontonasal suture to 1.0 cm above the nasal tip was made to provide exposure for a laterally based osteoplastic flap (1.0 mm $\times$ 2.5 mm) centered over the septum. After exposing the nasal cavity, bilateral submucoperichondrial flaps were elevated, and a 1.0 $\times$ 2.5-cm central basal segment of the septal cartilage was removed. The septum of the rabbit varies from approximately 0.25 mm to 0.75 mm in thickness.[6] Accordingly, caution was taken to avoid leaving minute pieces behind adherent to the perichondrium. Careful examination of the excised cartilage confirmed smooth, unfractured lateral borders. Both sides of the septal mucosa were then reapproximated, the osteoplastic flap closed, and skin incision sutured together. The rabbits received ad libitum access to food and water for an additional 208 days $\pm$ 35 days, or approximately 7 months, before being euthanized and removal of the remaining septum and perichondrium-covered defect. A representative specimen harvested after seven months in shown in Figure 1.

Specimens then underwent fixation, trimming of excess maxillary tissue, and standard histologic preparation, which consisted of immersion in neutral-buffered 10% formalin solution for 48 hours, followed by embedding in paraffin, sectioning, decalcification, and staining with hematoxylin & eosin. Slides were then examined under light microscopy. Optical micrometry was performed to measure the dimensions of any neocartilage islands found within the defect. An island of neocartilage was defined to be an isolated region of tissue consisting of chondrocytes and matrix within the defect area encased by perichondrium. Native septum was defined as cartilage appearing histologically consistent with typical mature cartilage and located superiorly in keeping with the location of the surgical remnant. The width and height of the islands were determined by micrometry and the greatest diameters for each island recorded. Slides judged to be of insufficient quality to accurately examine such that the key septal structures were obscured and were excluded from the study.

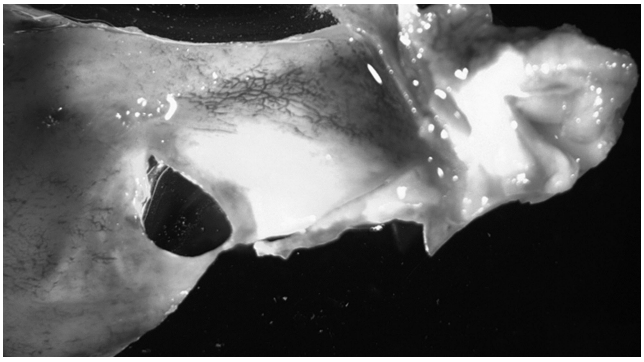

Fig. 1. Photograph of the cartilaginous nasal septum at harvesting, 7 months after partial submucosal resection. The columella is to the right. The defect is apparent in the center inferiorly as a translucent, avascular mucosal region.

RESULTS

All rabbits survived and tolerated the surgical procedure well. After histologic preparation, the set of slides obtained from one rabbit were found to be compromised as a result of plication of the sectioned specimen onto itself. These slides were therefore excluded from analysis. Sections from the remaining 16 rabbits were free of artifact, examined without difficulty, and found to have one to four islands of neocartilage each, or an average of 2.1 per animal. The majority of islands were discovered in the anterior inferior region. The width of the islands ranged from 50 to 475 μm with a mean of 190 μm, or approximately one third the width of the missing native septum in the defect area. The average neocartilage height was found to be 840 μm, of approximately one tenth the total defect height.

Morphologically, neocartilage was found to resemble native septal cartilage in terms of cell arrangement in the new matrix. Figure 2 is a coronal section of the rabbit nasal septum taken at 4$\times$ magnification showing both a remnant of the original septum superiorly (MatCh) as well as an island of neocartilage inferiorly (bracket). Both native septal cartilage and neocartilage exhibited chondrocytes in wide clusters toward the center of the cartilaginous zone with areas of closely spaced chondrocytes at the periphery. However, a neocartilage island generally consisted of several small, readily identifiable centers of proliferation (arrowheads) in contrast to the homogeneous native cartilage. Figure 3 shows native septal chondro-

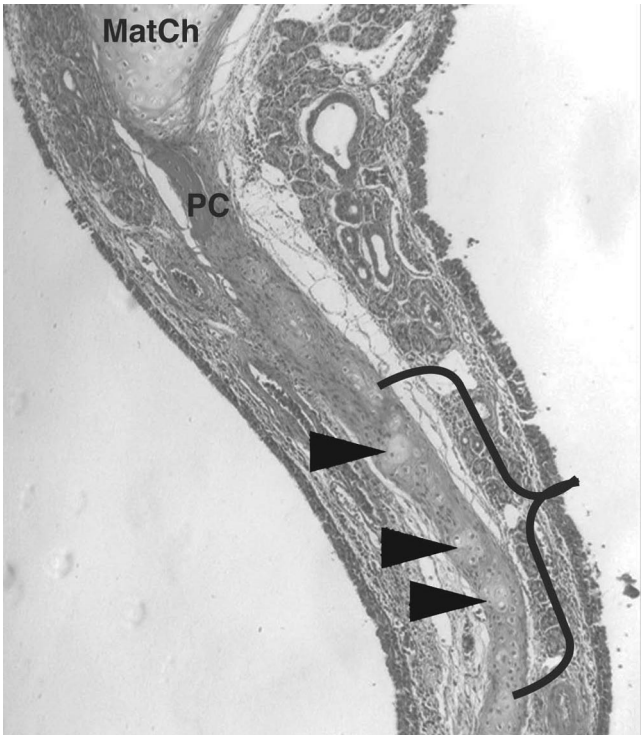

Fig. 2. Coronal section of the rabbit nasal septum taken at 4$\times$ magnification showing both a remnant of the original septum superiorly as well several centers of proliferating cartilage inferiorly (arrowheads). Perichondrium is also visible (PC). Mature cartilage is visible at the top of the image (MatCh).

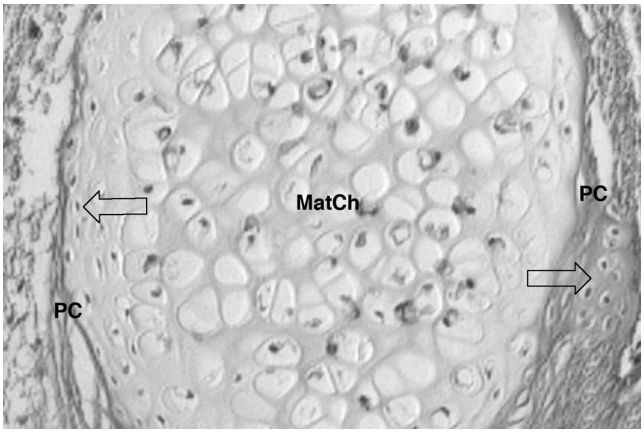

Fig. 3. Native septal cartilage from the same section shown in Figure 2, magnified to 10×. MatCh = mature chondrocytes; hollow arrow = peripheral chondrocytes with overlying perichondrium; PC = perichondrium.

cytes from the same section as Figure 2 at 10× magnification. The familiar cluster-like arrangement of vacuous-appearing, pale, round mature chondrocytes is evident here toward the center (MatCh). For comparison, a portion of the neocartilage island from the same section as Figure 2 is also depicted at higher magnification in Figure 4. Centers of proliferation are again indicated by arrowheads. Chondrocytes occur in dense clusters, reminiscent of the zones of proliferation found in epiphyseal growth plates. Nuclei are larger and cytoplasm darker, suggesting mitoses and active production of matrix, and overall cell shape is smaller and more elongated that that of the native septal chondrocytes.

DISCUSSION

Cartilage may undergo repair processes after trauma, albeit to a limited extent. Mao described hyaline cartilage as having a poor regenerative capacity, likely secondary to a paucity of chondrogenic cells,[1] and Silver et al. noted that in the setting of articular injury, fibrocartilage scar frequently

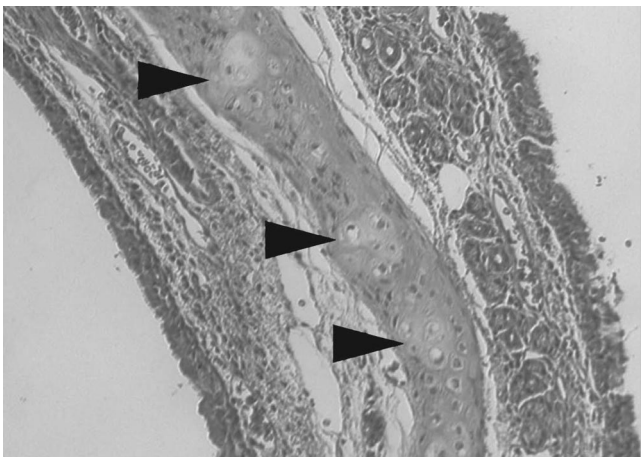

Fig. 4. Regenerated cartilage from the same section as shown in Figure 2, magnified to 10×. Arrowheads: islands of proliferation.

replaces damaged regions of hyaline.[3] The presence of multipotent mesenchymal stem cells in bone marrow has been exploited by orthopedic surgeons through the microfracture procedure for treatment of discrete articular cartilage defects. In this procedure, the base of the defect is repeatedly perforated through the subchondral bone, and a blood clot of marrow contents seeps into the defect. In various animal models, including primates, this clot is gradually replaced by chondrocytes, filling the defect to a far greater extent than that observed in untreated controls.[7] The cartilage formed after microfracture histologically resembles hyaline cartilage and contains more type II collagen (the predominant collagen found in hyaline cartilage) than controls.[8] Clinical studies in humans show magnetic resonance imaging and arthroscopic findings indicative of cartilage repair, and patients report functional joint improvement.[9]

Perichondrium, which covers most cartilaginous structures of the head and neck, but is absent on articular surfaces, is another well-known source of mesenchymal cells.[2] Verwoed-Verhoef et al. demonstrated that new cartilage is visible just 2 weeks after submucosal resections in young rabbits and may originate from perichondrial stem cells.[4] Histologic slides published in these studies closely resemble those we have produced in terms of morphology and extent of defect repair. Tcacencu found that cartilage repair after partial resection of the cricoid cartilage in adult rabbits only occurred in those areas in contact with a preserved perichondrial flap.[10] Pirsig et al. extended these findings to the clinical arena showing that in children, composite auricular grafts (skin and perichondrium) combined with demineralized bone scaffolds are capable of closing nasal septal perforations by substantial neocartilage growth as evidenced by biopsy.[11]

Nevertheless, to date, studies of nasal septal cartilage regeneration have largely been limited to young, immature model organisms followed for several weeks. Our results suggest that the perichondrium-lined nasal septum of adult rabbits, like that of immature animals, is capable of cartilage regeneration over a much longer survival timeframe, a period of 7 months. This scenario is clearly more relevant to the classic rhinoplasty or septoplasty patient.

The possibility exists, of course, that our findings are merely the product of repetitive surgeon's error, allowing microscopic remnants of septal cartilage to escape into the defect during the resection procedure. This seems unlikely, however, for a number of reasons. Meticulous steps were taken to ensure clear and intact removal of the cartilage grafts when creating the defect. The neocartilage exhibited morphologic differences from the native cartilage. Each of the 16 specimens exhibited similar neocartilage findings, and our results echo those of previous studies. Future experiments labeling proliferating tissues with radioactive isotopes may be necessary to definitively settle the question.

Several other issues still need to be resolved before the long-term goal of regenerating cartilage in the donor site can be realized. The mechanical behavior of cartilage is dependent on the biochemical makeup of the cartilaginous matrix. Whereas hyaline cartilage contains primarily type II collagen and is appropriate for the mechanical

function and structure of the septum, auricle, and articular surfaces, fibrocartilage, with mostly type I collagen, bridges ligament, tendon, and bone unions in the uninjured mammal.[3] After injury, fibrocartilage takes the place of hyaline in joint spaces, and its suboptimal mechanical properties lead to the development of osteoarthritis.[3] There are also differences even among the several sites of hyaline cartilage. Nasal septal cartilage has a biochemical makeup similar to articular cartilage in terms of water, collagen, and glycosaminoglycan content but has around 20% greater cellularity.[12] These qualities might contribute to the greater rigidity yet weaker tensile strength of nasal septal cartilage relative to articular.[13] Likewise, Tcacencu showed that proliferating cells in the defects of rabbit cricoid cartilage express alkaline phosphatase, an indication of future calcification.[10] It would be useful to perform immunohistologic staining and other assays on the neocartilage to ascertain the precise biochemical constituents.[12]

The issues of amount, rate, and type of neocartilage hinge centrally on the ability of various growth factors to infiltrate the damaged area. Although an exhaustive list of the humoral factors involved is beyond the scope of this article, members of the transforming growth factor β, insulin-like growth factor, fibroblast growth factor, bone morphogenic protein, and epidermal growth factor families have all been implicated in a variety of experimental animal models, along with platelet-derived growth factor and osteogenic protein 1.[14] One obvious means of exposing the perichondrial stem cells to the appropriate cytokine environment is implantation of a scaffold pretreated with the desired factors. Such scaffolds present the mesenchymal stem cells with a porous structure through which they can migrate, adhere, and receive nutrient diffusion as well as secure mechanical stability to withstand physiological stresses and strains without dislodging. In addition, the optimal scaffold degrades slowly to inert products within the body, allowing gradual replacement by regenerating cartilage.[14,15] Scaffolds constructed from fibrin glue, collagens, hyaluronic acid, polysaccharide hydrogels such as agarose, alginate, and chitosan, and various synthetic polymers, including, most notably, polylactic acid and polyglycolic acid, have all been tried in vivo with varying degrees of success.[15] Table I lists some of the current U.S. Food and Drug Administration (FDA)-approved materials that might serve the dual purpose of both cytokine vehicle and scaffold as potential filler materials for SMR defects, although the FDA indications vary from product to product.

Clinically, the defects that result from harvesting of the cartilaginous nasal septum are not replenished. Once accessed, this reservoir is considered exhausted. However, our results in rabbits suggest that some degree of regeneration may occur and might be exploited to replenish this reservoir. The perichondrium is a rich source of stem cells and is capable of seeding defects with dividing cells. The neocartilage is influenced to proliferate and differentiate after exposure to various cytokines and scaffolds. When translated to the humans, these observations imply that one may create a septal defect in the process of harvesting, implant a scaffold with or without appropriate cytokines, and return later to harvest regenerated cartilage. Studies in the orthopedic literature suggest this occurs in articular cartilage—despite the sparse vasculature and lack of perichondrium—after microfracture, which functions in a manner analogous to nasal septal perichondrium by allowing migration of mesenchymal stem cells into the defect. Therefore, it is not unreasonable to expect similar or better outcomes in the nasal septum where the physiological and anatomic environment is much more inviting. This is a focus of our future investigation.

TABLE I.
Potential Scaffolds for Submucosal Resection (SMR) Defects.

	Scaffold	Consists of
Preformed	Ethisorb (Johnson and Johnson, New Brunswick, NJ)	PLA and PGA
	Alloderm (LifeCell, Branchburg, NJ)	Human acellular dermal matrix
	Bio-Gide (Geistlich Biomaterials, Zurich, Switzerland)	Porcine type I/III collagen
	Fasciablast (Fascian Biosystems, Beverley Hills, CA)	Human fascia lata
	Gelfoam sponge (Pfizer, New York, NY)	Porcine dermal gelatin
	Autologous temporalis fascia	
	Autologous fascia lata	
Injectable	Tisseal (Baxter Healthcare, Deerfield, IL)	Fibrin glue
	Hyalaform (INAMED, Santa Barbara, CA)	Chicken hyaluronic acid
	Restylane (Q-Med, Uppsala, Sweden)	Recombinant hyaluronic acid
	Zyderm/Zyplast (INAMED, Santa Barbara, CA)	Bovine collagen
	Cosmoderm/Cosmoplast (INAMED, Santa Barbara, CA)	Human collagen
	Cymetra (LifeCell, Branchburg, NJ)	Injectable form of Alloderm
	Fascian (Fascian Biosystems, Beverley Hills, CA)	Injectable form of Fasciablast

PLA = polylactic acid; PGA = polyglycolic acid.

CONCLUSION

After partial submucosal resection of the cartilaginous nasal septum in the mature rabbit, cartilage may regrow substantially in the defect covered by perichondrium. This regrowth occurs in skeletally mature rabbits, becomes quickly histologically apparent, and persists indefinitely. The neocartilage morphologically resembles but is not identical to native septal cartilage. Several cytokines have been demonstrated in cartilage healing, and many scaffolds that have been shown to encourage chondrocyte growth and differentiation are already clinically available. In the future, our studies will focus on how to optimize nasal septal cartilage regeneration with scaffold implantation and the proper spatiotemporal application of growth factors to create a renewable supply of hyaline cartilage within the nasal septum.

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