Age-Related Histologic Changes in Human Nasal Cartilage

Judy W. Lee, MD; Jonathan McHugh, MD; Jennifer C. Kim, MD; Shan R. Baker, MD; Jeffrey S. Moyer, MD

The 3 primary donor sites for autologous cartilage graft material in rhinoplasty and nasal reconstruction are the nasal septum, ear, and rib. Nasal septal cartilage is most commonly used owing to its ease of harvest within the surgical field without significant additional donor site morbidity. The thickness, flat contour, and durability of nasal septal cartilage are favorable qualities for long-term strength and support. Although septal cartilage is often the preferred choice in grafting material, its use may be limited by availability, particularly in noses that were previously operated on or traumatized. To overcome this problem, more recent efforts in tissue engineering have been directed toward creating new cartilage in vitro and in vivo using mesenchymal stem cells and progenitor cells seeded onto scaffolds.¹

Age-related changes in the chemical composition of human cartilage are well documented. Much of the information on this subject has been derived from studies on articular cartilage. Aging has been studied extensively in normal and osteoarthritic joint cartilage, where significant decreases in proteoglycan content are accompanied by mechanical weakening of the cartilage.² Although they are associated with aging, it remains unclear whether these findings are specific for degenerative and osteoarthritic cartilage rather than the normal aging process. As a result, it remains difficult to extrapolate this data to nasal cartilage, which does not undergo the same degree of mechanical stress in the nose.

The notion that the quality and strength of nasal cartilage declines with advancing age is a commonly accepted be-
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Objective of our study was to characterize the cellular and architectural changes in human nasal septal cartilage that occur with aging in healthy patients.

Methods

Experimental Design

This study was approved by the institutional review board of the University of Michigan Medical School. Nasal septal cartilage was harvested from 50 consecutive patients who underwent septrhinoplasty, rhinoplasty, or septrhinoplasty between October 25, 2011, and April 10, 2012. Patients with a history of granulomatous or autoimmune disease, sinonasal tumors, head and neck radiation therapy, chronic rhinosinusitis, revision surgery, or intranasal drug abuse were excluded from the study. Demographic information, including age and sex, was obtained from the medical record.

Cartilage Samples

Harvested cartilage samples were fixed in 4% formalin immediately after harvest and subsequently decalcified in 10% formic acid for 10 days. The decalcified samples were rehydrated using 70% to 100% ethanol, rinsed with xylene, and embedded in paraffin. Cartilage segments adherent to bone were discarded before embedding. Each specimen was sectioned in 5-μm sections and stained with hematoxylin-eosin (H&E) and safranin O. The cartilage samples were stained with safranin O to highlight the concentration distribution of proteoglycan content, which is composed of sulfated glycosaminoglycan (GAG) chains, including chondroitin sulfate and keratan sulfate.17 Intense red staining of cartilage with safranin O is due to high proteoglycan content representing active chondrocyte activity, whereas decreased staining occurs with a reduction in proteoglycan content within the cartilage matrix. Calcified cartilage is demonstrated by light pink staining.

Histologic Scoring

Fifty sections of nasal septal cartilage were analyzed. All samples were masked with respect to donor age and examined by an experienced pathologist (J.M.). A modified version of the Mankin grading scale, a well-described histologic scoring system for the quality of articular cartilage, was used to score each nasal cartilage sample.18-19 Scored features for H&E staining included irregular perichondrium, organization, chondrocyte clusters, perichondrium fibrosis, chondrocyte necrosis, and fibrinoid degeneration, with a maximum possible score of 16 (Table). A separate score was determined for safranin O staining, with a maximum possible score of 3. Two scores were determined for each patient sample, including a cumulative score for all H&E staining features and a score for safranin O staining.

Statistical Analysis

Correlation between histologic score and age was determined by calculating the correlation coefficient (R²) using a scatterplot. Statistical significance was determined for all correlations with linear regression analysis. Statistical analyses were performed using SPSS software, version 19.0 for Windows (IBM). Differences were considered to be statistically significant at P ≤ .05.

Results

Among the 50 patients, 22 were female and 28 were male; their ages ranged from 17 to 79 years (mean [SD], 36.5 [14.1 years]). The mean (SD) cumulative H&E score for all patients was 2.66
(1.29), and the mean safranin O score was 0.56 (0.81). With regard to sex, the mean (SD) cumulative H&E score for female patients was 2.48 (1.81) compared with 2.81 (1.18) for male patients \((P = .36)\). The mean (SD) safranin O scores for female and male patients were 0.65 (0.78) and 0.48 (0.85), respectively \((P = .46)\). Histopathologic evaluation of the H&E-stained samples did reveal some differences in chondrocyte cellularity between younger and older patients, but these findings were not consistent throughout the sample group. Increased cellularity is commonly seen with cartilage injury, causing an influx of chondrocytes, which can then form abnormal clusters of cells. Figure 1 represents the H&E sample from a 79-year-old patient, which demonstrates slight hypercellularity compared with the sample from an 18-year-old patient with normal chondrocyte cellularity (Figure 2). Safranin O staining for cartilage matrix, however, did vary with patient age, with decreased staining seen with advancing age, representing reduced proteoglycan content within the cartilage matrix and decreased chondrocyte activity. Figure 3 demonstrates normal, intense safranin O staining in the same 18-year-old patient, in contrast to the reduced staining seen in the 79-year-old patient (Figure 4).

There was no significant correlation between increasing age and H&E score \((P = .19)\), as seen in Figure 5, but there was a statistically significant correlation between age and safranin O staining score (Figure 6). Higher safranin O staining scores, representing reductions in cartilage proteoglycan content within the cartilage matrix and overall decreased chondrocyte activity, were seen with increasing age \((P = .01)\). A linear regression of best fit was determined as follows:

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\text{Safranin O staining score} = (0.02 \times \text{Age [in years]}) - 0.17, \\
\text{with a correlation coefficient } (R^2) \text{ of } 0.121.
\]

With this regression, a safranin O score may be calculated as a dependent variable of age. A preoperative safranin O score equal or close to zero suggests a normal, or near-normal, cartilage matrix architecture with appropriate active chondro-
cyte activity for a given patient. Safranin scores of 1 or higher suggest abnormally decreased proteoglycan content with corresponding reductions in chondrocyte activity. With regard to sex, scatterplot analyses of H&E and safranin Oscores for female and male patients did not show significant correlations with donor age (Figures 7, 8, 9, and 10).

Comment

During the aging process, the nose undergoes significant anatomic changes, including changes in skin quality, structural alterations in the underlying bone framework and nasal cartilages, and progressive shifts in the nasal airways. The nasal skin is thinner and less elastic in elderly persons, making it more prone to injury. A cadaveric study on surgical anatomy of the elderly nose by Vacher and colleagues demonstrated that the primary morphologic change seen with aging was nasal tip ptosis due to cutaneous atrophy and weakness of the tip muscles from fat infiltration. Although the upper two-thirds of the nasal skin becomes increasingly thin with aging, the tip and alar skin thicken owing to an increase in the number of sebaceous glands. Age-related morphologic changes are the most dramatic in the lower third of the nose, with elongation of the lateral nasal walls and dropping of the nasal tip caused by thinning of the nasal skin envelope, separation of the attachments between the upper and lower lateral cartilages, and weakening of the nasal cartilages. Nasal cartilage tends to ossify and become brittle or to soften excessively, losing support. Weakening of the suspensory ligaments in the scroll region further exacerbates alar cartilage collapse. These findings can result in overall lengthening of the nose and loss of tip support, which can contribute to the progressive nasal obstruction commonly seen in older patients.

These clinical changes must be considered when one is planning for rhinoplasty or nasal reconstructive surgery in elderly patients, particularly when cartilage grafts may be required for aesthetic and/or functional purposes. Cartilage grafts, including spreader, batten, and strut grafts, are often used during rhinoplasty to strengthen internal and external nasal valve dysfunction in addition to increasing tip projection and rotation. Although it is generally believed that nasal cartilage quality decreases with advancing age, with variable degrees of ossification and/or weakening, there are few studies to support this hypothesis.
There are several distinct age-dependent patterns of growth activity within the cartilaginous nasal septum. The anterior free end typically has the highest degree of growth activity throughout an individual’s lifetime.21-23 Early studies by Vetter and colleagues21-23 demonstrated high levels of chondroitin sulfate incorporation and chondrocyte density in the anterior free end of the septum in children, adolescents, and adults. This was in contrast to the cartilage present in the caudal, posterior, and floor regions of the septum, which showed high growth activity only during childhood, with progressive decline into adulthood. A cadaveric study assessing the anatomy of the nasal septum and identifying variations in thickness found that the central and floor regions of the septum were significantly thicker than the anterior and caudal ends.24 Therefore, it was recommended that cartilage grafts used during rhinoplasty be harvested from this central or floor region whenever possible.

This remains problematic, however, because the central regions of the septum have demonstrated the least amount of chondrocyte density and activity with advancing age. The decline of available septal cartilage for grafting with aging was further demonstrated in a radiologic study by Kim et al,25 wherein sagittal magnetic resonance imaging views of 280 random healthy patients of all ages revealed that the maximum area and proportion of cartilage to the total septal area progressively declined after age 30 years. These findings support the fact that the quality and availability of harvestable septal cartilage becomes an increasing problem with aging, and the surgeon should be aware of these age-related limitations when cartilage grafting is considered.

Age-related changes in the biochemical composition and mechanical properties of human nasal septal cartilage have been described by Rotter and colleagues.15 Human nasal septal cartilage specimens were subjected to sequential loading increments to calculate stress-strain relationships using the equilibrium modulus and hydraulic permeability, and the findings showed that nasal septal cartilage is less stiff and more forgiving of mechanical stress than articular cartilage. With respect to age, Rotter et al15 found that compressive equilibrium modulus, hydraulic permeability, and GAG content were dependent on donor age. Nasal cartilage was stiffer and weaker with advancing age, which was postulated to be due to fibrotic changes to the cartilage matrix.

With limited availability and age-related changes that affect nasal septal cartilage, there have been increasing efforts to develop tissue-engineered septal cartilage. However, recent studies on this topic have had conflicting findings, with some showing favorable stability and composition and others showing warping, ossification, decreased chondrocyte activity, and foreign body reaction.16,26-29 Rotter and colleagues16 reported that tissue-engineered septal cartilage developed from human septal chondrocytes seeded onto scaffold discs and implanted subcutaneously into athymic mice showed similar biochemical and biomechanical properties independent of donor age. Analyses for GAG and hydroxyproline content of this engineered cartilage did not show any difference with age, despite distinct reductions in GAG seen in the native donor cartilage from older patients (aged >40 years). Moreover, the biomechanical properties of the engineered cartilage remained similar between age groups, with increasing mechanical stability over time. Overall, the matrix composition and stiffness of the engineered cartilage did not vary with donor age.

In contrast, Christophel and colleagues29 showed that engineered cartilage transplants of rabbit chondrocytes grown on established synthetic scaffolds lost their 3-dimensional shape, with a significant decrease in mass and chondrocyte viability. Interestingly, they also reported progressive formation of bone and foreign body reaction with these constructs. With such inconsistency in the scientific literature regarding tissue-engineered cartilage, its use remains investigational at this time, mainly in animal models, but it may provide a viable alternative for cartilage grafting in the future, particularly in the aging population.

The general pattern of cartilage weakening with age is consistent with studies of the aging process in human articular cartilage. Studies in the osteoarthritis literature have reported altered transforming growth factor β signaling in aged and osteoarthritic cartilage.30 Chondrocytes in aged cartilage are...
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Although nasal cartilage does not endure the same degree of mechanical stress as articular cartilage, many of the same changes in biochemical composition, extracellular matrix, and chondrocyte activity occur with aging in the 2 tissue types. As a result, we have used a modified version of the Mankin histologic scoring system in the present study to compare nasal septal cartilage with aging. The cumulative H&E scores were not significantly correlated with advancing age, but there was a trend toward increased chondrocyte cellularity and clustering, commonly seen with cartilage injury, in the older donor patients. Furthermore, we found a statistically significant positive correlation between safranin O scores and age. Higher safranin O scores seen with advancing age represent abnormal reductions in the proteoglycan content found within the cartilage matrix and is a measure of decreased chondrocyte activity. These results are consistent with the biomechanical and biochemical findings reported by Rotter and colleagues. Sex did not seem to affect either H&E or safra-

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Analysis and interpretation of data: Lee, McHugh, and Moyer.

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Critical revision of the manuscript for important intellectual content: McHugh, Kim, and Baker.

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Study supervision: McHugh, Baker, and Moyer.

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REFERENCES
24. de Pochat VD, Alonso N, Figueredo A, Ribeiro EB, Mendes RR, Meneses JV. The role of septal cartilage in rhinoplasty: cadaveric analysis and

jamafacialplasticsurgery.com


