A Compositional Analysis of Human Nasal Septal Cartilage

Mark R. Homicz, MD; Kevin B. McGowan, BSE; Lisa M. Lottman, BS; Gordon Beh, BS; Robert L. Sah, MD, ScD; Deborah Watson, MD

Background: Nasal septal cartilage is well established as an autograft material. Tissue engineering methods are now being developed to synthesize cartilage constructs with the properties of this type of cartilage. However, important baseline data on the composition of native septal cartilage is sparse.

Objectives: To characterize quantitatively the major biochemical constituents of native adult human septal cartilage and determine age- or sex-related variation in composition.

Methods: Cartilage was harvested from the inferior region of the nasal septum in 33 patients (mean±SD age, 47.0±13.5 years; range, 24-80 years) during routine septoplasty or septorhinoplasty. Biochemical assays were used to determine the quantities, relative to wet weight, of the major constituents of cartilage: water, collagen (from hydroxyproline), sulfated glycosaminoglycan (sGAG), and chondrocytes (from DNA).

Results: On average, each gram of wet cartilage contained 77.7% water, 7.7% collagen, 2.9% sGAG, and 24.9 million cells. Hydration and collagen content showed no significant age variation. Advancing age was associated with a reduction in sGAG content (7.7% per decade, P=.02) and cellularity (7.4% per decade, P=.05). No significant sex differences were found in any of these cartilage constituents.

Conclusions: This study represents the first biochemical characterization of the composition of native human septal cartilage. The data serve as a baseline for future comparison of the properties of tissue-engineered neocartilage constructs. Furthermore, the age-associated variations in cartilage composition have implications for patient selection for reconstructive procedures.

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Head and neck reconstructive surgeons are often faced with the need for structural components for the reconstruction of cartilaginous deficits created by trauma, tumor resection, or congenital malformations. Autografts of auricular, costal, and nasal cartilage frequently are used to provide the structural support during reconstructive procedures. However, several factors limit their utility: finite amounts of cartilage are available, surgical harvest of cartilage grafts can be associated with significant morbidity, and some cartilage grafts possess a suboptimal geometric structure for defect reconstruction.

These limitations have prompted investigators to search for alternatives to cartilage autografts for structural support. Currently, tissue engineering methods are being used to create experimental neocartilage by seeding cultured chondrocytes onto preformed polyglycolic acid scaffolds. By alteration of scaffold geometry before seeding, cartilage grafts can be created in defined shapes and sizes. This approach allows for cell expansion in culture, potentially providing a large amplification of chondrocytes from harvested cartilage for use in tissue engineering of autologous neocartilage.

Although donor chondrocytes can be obtained from several different anatomic locations, nasal septal cartilage represents an ideal material for tissue engineering. This type of cartilage is easily harvested with minimal morbidity and without functional or cosmetic deficits. Moreover, septal cartilage in its native form has superior structural rigidity when compared with the more elastic auricular cartilage, making it the current preferred autograft material for mechanical support during reconstructive surgery. These structural properties, attributable to the unique composition of extracellular matrix (ECM) components synthesized by...
septal chondrocytes, could potentially be reproduced in neocartilage grafts made from these cells.

Promising initial work in tissue engineering has produced septal chondrocyte–derived grafts that demonstrate histologic characteristics of cartilage when grown in vitro or reimplanted into animal models. One approach to engineering neocartilage constructs that resemble native cartilage is to compare these cartilage constructs with native cartilage in terms of biochemical composition. However, the basic biochemical properties of native septal cartilage currently are not well characterized.

In general, adult cartilage is composed of relatively few cells, which are embedded in an extensive ECM. The ECM components are synthesized and secreted by chondrocytes. The most abundant molecular component by weight in cartilage is collagen. In articular cartilage, type II collagen and other less common collagenous form fibrils in the ECM, which are largely responsible for the tensile strength of cartilage. Within each collagen molecule, residues of the amino acid proline become enzymatically hydroxylated during synthesis. Since relatively few other molecules undergo this modification, measurement of hydroxyproline serves as a reliable method in many tissues for quantifying collagen.

The other major molecular constituent of cartilage is proteoglycan, predominantly aggregan. Aggrecan is a macromolecule that consists of many sulfated glycosaminoglycan (sGAG) chains attached to a protein core. The high concentration of acidic carboxyl and sulfate groups on glycosaminoglycan side chains are ionized at physiologic pH, giving a high density of negative charge that attracts positive ions and water into the ECM. This propensity of proteoglycans to swell with fluid and the restraining function of collagen are responsible for the unique mechanical properties of cartilage.

Because of its importance in osteoarthritis research, human articular cartilage has been the focus of a large body of scientific work. The relative quantities of human articular cartilage components vary with maturation and normal aging, pathologic degeneration, and joint location. The cellularity (cell number per tissue volume) of articular cartilage decreases slightly during postmaturation aging and/or degeneration. Since the density of normal adult cartilage is fairly constant, approximately 1.1 g/cm³ of wet tissue, wet weight is often used as a normalization variable for cartilage components. The sGAG content and charge density of cartilage from the knee joint are relatively stable during normal aging but are decreased with degeneration. The collagen content of cartilage seems stable during aging but becomes disorganized (and diminished on a wet weight basis) with degeneration.

Given the structural support (but only modest weight bearing) provided by nasal septal cartilage, its biochemical makeup may vary significantly from articular cartilage. Before the recent advances in tissue engineering, there was little clinical interest in characterizing the major components of human septal cartilage. Consequently, this tissue has not undergone a complete compositional analysis. This study begins the process of quantifying human septal cartilage constituents by determining the cell density and the content of water, collagen, and sGAG present in septal cartilage. Because of possible implications for patient selection for tissue engineering, age- and sex-based variations in cartilage makeup are examined in particular.

**Human septal cartilage was obtained from 33 patients undergoing septoplasty or septrhinoplasty. Only specimens taken from the inferior region (along the maxillary crest) of the quadrangular cartilage were included in this study to avoid inaccuracies due to regional variation in tissue composition. All tissue collection was approved by the institutional review boards of the University of California, San Diego, and the San Diego Veterans Affairs Hospital.**

After surgical harvest, specimens were placed in isotonic sodium chloride solution at 4°C and transported to the laboratory. Cartilage specimens were sectioned for individual processing and weighed wet. Following lyophilization for 16 to 20 hours, the dry weight of each section was determined. Water content of each sample was obtained by subtracting dry weight from wet weight and dividing this quantity by wet weight.

Specimens were then digested with a solution of 0.5 mg/mL of Proteinase-K (Roche Diagnostics, Indianapolis, Ind) in 100 mM phosphate and 5 mM EDTA (pH 7.1) at 60°C for 16 hours. Digests were stored at −70°C until use. Digest solutions underwent the following biochemical analysis.

A spectrophotometric assay for sGAG was performed using the 1,9-dimethylmethylene blue (DMMB) dye reaction. Duplicate 40-µL aliquots of each digest solution were assayed with the DMMB dye. Absorbance at 525 nm was measured on a spectrophotometric plate reader and compared with a plot of standards made from shark chondroitin sulfate type C (C-4384; Sigma-Aldrich, St Louis, Mo).

DNA content of the digests was measured as previously described by Kim et al. Briefly, the fluorescence (excitation, 365 nm; emission, 458 nm) was measured for mixtures of 100 µL of each digest in 1 mL of 10 mM Tris, 1 mM EDTA, and 200 mM of sodium chloride (TEN, pH 7.5) with and without 0.1 µg/mL of Hoechst 33342 dye (B-2883; Sigma-Aldrich) using a spectrofluorometer (F-2000 Fluorescence Spectrophotometer; Hitachi, San Jose, Calif). In particular, to account for the intrinsic tissue autofluorescence of human cartilage, the fluorescence of the tissue digest and TEN solution was subtracted from that of tissue digest, TEN, and Hoechst 33342 dye to obtain the fluorescence attributable to DNA. Samples were assayed in duplicate. Calf thymus DNA (D-1301; Sigma-Aldrich) was used as a standard. The cell content of each digest was calculated based on the estimated value of 7.7 pg of DNA per chondrocyte.

Finally, the content of hydroxyproline was quantified using the chloramine T reagent/p-dimethylaminobenzaldehyde (DMBA) assay. Duplicate 100-µL aliquots of each digest were hydrolyzed with equal volumes of 12N hydrochloride at 110°C for 18 hours. After evaporation of hydrochloride, specimens were reacted with chloramine T and DMBA at 60°C for 20 minutes and then cooled. The absorbance of the assay solutions at a wavelength of 560 nm was measured in a spectrophotometric plate reader and compared with that of standards made from 1,4-hydroxyproline (H-6002; Sigma-Aldrich) to determine hydroxyproline content. The content of hydroxylated proline residue was used to estimate collagen content of the cartilage using the conversion factor of 1 g of hydroxyproline per 7.1 g of collagen.  

Statistical analysis was conducted using Systat software (Systat Inc, Evanston, Ill). Linear regression analysis was used to analyze age-related trends in hydration, cellularity, sGAG content, and collagen content. Regression slope was ex-
pressed as percent change per decade relative to the value at 20 years of age. A 2-tailed t test was used to compare the slopes between regression lines for men and women. Analysis of variance (ANOVA) was used to assess the independent effects of age groups (20-40 years, 41-60 years, and 61-80 years) and sex on hydration, cellularity, sGAG content, and collagen content. The limited number of patient samples in the oldest age group did not allow a fully factorial ANOVA to determine interactive effects of age and sex. Data are presented as mean ± SE for groups and mean ± SE for slopes of regression curves.

Table 1. Sex Variation in Septal Cartilage Components*

<table>
<thead>
<tr>
<th>Patients</th>
<th>Mean Age, y</th>
<th>Hydration, %</th>
<th>Collagen, mg/g WW</th>
<th>sGAG, mg/g WW</th>
<th>Cells, 10⁶/g WW</th>
</tr>
</thead>
<tbody>
<tr>
<td>All patients</td>
<td>47.0 ± 13.5</td>
<td>77.7 ± 1.9</td>
<td>87.1 ± 20.1</td>
<td>28.7 ± 9.2</td>
<td>24.9 ± 9.1</td>
</tr>
<tr>
<td>Men (n = 22)</td>
<td>47.8 ± 14.1</td>
<td>77.3 ± 1.9</td>
<td>88.7 ± 21.4</td>
<td>29.5 ± 9.3</td>
<td>24.8 ± 9.8</td>
</tr>
<tr>
<td>Women (n = 11)</td>
<td>45.5 ± 12.9</td>
<td>78.3 ± 1.7</td>
<td>83.9 ± 17.8</td>
<td>27.2 ± 9.4</td>
<td>25.2 ± 8.1</td>
</tr>
<tr>
<td>P value</td>
<td>.64</td>
<td>.23</td>
<td>.52</td>
<td>.36</td>
<td>.91</td>
</tr>
</tbody>
</table>

Table 2. Variation in Septal Cartilage Components by Age Grouping*

<table>
<thead>
<tr>
<th>Patients</th>
<th>Mean Age, y</th>
<th>Hydration, %</th>
<th>Collagen, mg/g WW</th>
<th>sGAG, mg/g WW</th>
<th>Cells, 10⁶/g WW</th>
</tr>
</thead>
<tbody>
<tr>
<td>Young adult (n = 12)</td>
<td>33.7 ± 3.9</td>
<td>78.4 ± 1.7</td>
<td>88.6 ± 20.8</td>
<td>30.7 ± 11.5</td>
<td>27.6 ± 9.1</td>
</tr>
<tr>
<td>Middle-aged adult (n = 17)</td>
<td>50.2 ± 5.4</td>
<td>77.1 ± 2.0</td>
<td>89.7 ± 19.5</td>
<td>29.6 ± 6.9</td>
<td>25.4 ± 9.6</td>
</tr>
<tr>
<td>Aged adult (n = 4)</td>
<td>73.5 ± 5.8</td>
<td>77.8 ± 0.7</td>
<td>77.4 ± 23.1</td>
<td>19.2 ± 5.0</td>
<td>15.1 ± 10.0</td>
</tr>
<tr>
<td>P value</td>
<td>&lt;.001</td>
<td>.22</td>
<td>.55</td>
<td>.07</td>
<td>.06</td>
</tr>
</tbody>
</table>

Abbreviations: g WW, gram of cartilage wet weight; sGAG, sulfated glycosaminoglycan.

A portion of the quadrangular septal cartilage was harvested from 33 patients (22 men and 11 women). Patient age ranged from 24 to 80 years (mean ± SD age, 47.0 ± 13.5 years). The age of male patients (47.8 ± 14.1 years) and female patients (45.5 ± 12.9 years) was similar (P = .64). Table 1 and Table 2 present the mean quantities of septal cartilage constituents for different sex and age groups.

Mean hydration per patient ranged from 73.9% to 81.4%. The average water content of cartilage for all patients was 77.7%. Regression analysis showed no age-related trend in hydration. Figure 1 demonstrates the lack of significant age variation in collagen content by linear regression [slope = −0.23 ± 0.11 mg of collagen/(gram of wet weight × year)] (P = .65). Also, no sex difference (P = .41) was found in the slopes of collagen vs age curves [0.006 ± 0.34 and −0.49 ± 0.43 mg of collagen/(gram of wet weight × year)] for men and women, respectively. Furthermore, by ANOVA analysis, collagen content was not statistically different between age groups (P = .55) or sexes (P = .52).

The average amount of sGAG in the cartilage specimens was 28.7 mg (range, 13.6-44.6 mg) per gram of cartilage wet weight. Figure 3 shows the effects of advancing age on sGAG content. The amount of sGAG present in septal cartilage decreased with advancing age (P = .02) by linear regression. Overall, sGAG decreased by 7.7% per decade [slope = −0.28 ± 0.11 mg/(gram of wet weight × year)]. These age-related changes were not significantly different (P = .95) for men and women [slope = −0.30 ± 0.13 and −0.28 ± 0.22 mg/(gram of wet weight × year), respectively]. Additionally, ANOVA showed a tendency for an age-related change in sGAG content with aging (P = .07) but not with sex (P = .36).

Wet cartilage was found on average to contain 24.9 million cells per gram of wet weight. Cellularity varied widely from 3.7 million to 51.8 million cells per gram of wet weight. As patient age increased (Figure 4), cellularity decreased by 7.4% per decade by regression analysis [slope = −0.23 ± 0.11 million cells/(gram of wet weight × year)].

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weight $/H_11003\text{year}\} (P=0.05)$. Of interest is that this age dependence was somewhat stronger in women, who exhibited a 13.2% decrease per decade $[\text{slope}=-0.50\pm0.13$ million cells/(gram of wet weight $/H_11003\text{year}\}] (P<0.005)$. Men, in contrast, had only a 4.3% decrease per decade $[\text{slope}=-0.006\pm0.34$ million cells/(gram of wet weight $/H_11003\text{year}\}] (P=0.44)$. However, the sex variability of these age-related changes in cellularity was not significant $[P=0.14]$. Also, cellularity showed a strong tendency to be affected by age group $[P=0.06]$ but not sex $[P=0.91]$ by ANOVA.

**COMMENT**

Although the composition of articular cartilage is well documented, to our knowledge no previous studies have quantified the cellularity and major matrix components of human nasal septal cartilage. To provide baseline data for comparison of neocartilage engineered from human septum, this study sought to establish such information using 33 adult septal cartilage specimens. Only cartilage taken from the inferior strip of septum (just superior to the maxillary crest) was used in this study because septal location is a factor that likely affects cartilage constitution. This assumption is supported by the substantial regional variation encountered in articular cartilage, which demonstrates distinct composition in different joints, in separate locations of the same joint, and at varying depths from the joint surface.

The biochemical assays used in this study to quantify cartilage components are also based on several assumptions that merit discussion. The calculation of collagen content from measured hydroxyproline assumes a constant mass ratio of 7.11 g of collagen for every gram of hydroxyproline. This relation is based on measurements of hydroxyproline content in purified collagen prepared by pepsin digestion of human articular cartilage.\(^2^2\) The use of this ratio thus assumes that the purification method did not selectively alter the ratio of hydroxyproline to collagen. The conversion factor of 7.11 is consistent with studies that have measured this ratio in bovine cartilage.\(^2^3\)-\(^2^5\)

The DMMB assay for sGAG content compares the absorbance of experimental samples with that of known concentrations of sGAG in standard solutions. The sGAG chondroitin sulfate was used as the standard in this experiment. Chondroitin sulfate represents most (70%-96%) of the sGAG present in aggrecan and articular cartilage, with hyaluronan and keratan sulfate comprising smaller portions.\(^2^6\) Thus, use of chondroitin sulfate provides a reasonable standard source for comparison. In addition, the determination of sGAG in this way is highly correlated with fixed-charge density in human articular cartilage.\(^1^7\)

Finally, the use of Hoechst 33258 assay for DNA determination from human cartilage requires care owing to intrinsic tissue autofluorescence. Significantly higher autofluorescence has been noted in human articular cartilage compared with bovine tissue.\(^2^1\) New evidence suggests that septal cartilage also demonstrates substantial intrinsic fluorescence.\(^2^7\) To account for this confounding variable, autofluorescence of samples without Hoechst 33258 dye was measured and used to correct the measured fluorescence of samples combined with Hoechst dye.

Given the limitations thus stated, this study provides the most extensive quantitative analysis of human nasal septal cartilage composition to date. On average,
each wet gram of cartilage contained 77.7% water, 7.7% collagen, 2.9% sGAG, and 24.9 million cells. The quantities of ECM components compare with those of cartilage obtained from human femoral condyle, which contains 65% to 75% water, 15% to 20% collagen, and 3% to 6% sGAG. The measured cellularity is somewhat higher than what has been reported for human articular cartilage, with values of 18 million cells/cm³ for midradial zone adult human articular cartilage, 29-14 million cells/cm³ for septum. However, since enzymatic digestion of cartilage from several different locations on the nasal septum using trypsin and collagenase digests. Their isolation method yielded between 0.5 and 7.5 million cells per gram of wet cartilage, depending on septal location and patient age. The authors concluded that the highest cell numbers and greatest cell viability could be obtained from digests of the anterior and central portions of the septum. However, since enzymatic digestion of cartilage leads to a significant degree of chondrocyte degradation and mortality, such studies are unlikely to provide an accurate representation of the cell density in native septal cartilage.

To examine whether certain patient characteristics could alter the effectiveness of a septal cartilage graft used for tissue engineering, this study looked for any age- or sex-related variance in composition. No statistically significant sex differences in quantities of any cartilage constituents were found by ANOVA or regression analysis. In contrast, both regression analysis and ANOVA showed either significant decrease or a strong tendency toward decrease in cellularity and sGAG content with advancing age. The lack of statistical significance in the ANOVA analysis is likely due to the small number of patients (n=4) in the oldest ANOVA group (age range, 61-80 years).

The exact implications of these age-related trends on tissue engineering methods remain to be established. Based on ongoing studies, the cell numbers obtained from septal cartilage from elderly patients are still large enough to afford ample numbers of cells for culture and subsequent modulation. Whether or not the cells from aged patients exhibit similar viability and metabolic activity to cells from younger individuals remains to be seen. The decrease in sGAG content associated with age, which has been demonstrated herein, may be an indicator of diminishing synthetic capacity but alternatively could be due to accelerated catabolic activity in chondrocytes from older individuals.

In conclusion, tissue-engineered cartilage will likely serve a role in future reconstructive surgery of the head and neck. A major current limitation of this technology is a lack of data on native septal cartilage composition to serve as a baseline for comparison to engineered neo-cartilage. To begin to fill this information gap, a compositional analysis of human nasal septum was presented in this article. Future experimental work will be necessary to broaden the scope of knowledge on the biochemical composition and mechanical function of native human septal cartilage.

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