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A Compositional Analysis of Cadaveric Human Nasal Septal Cartilage

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Abstract

Objectives—1) To localize quantitatively the major biochemical constituents of native adult human septal cartilage across whole septa.

Study Design—Prospective, basic science

Methods—The nasal septa from seven cadavers were partitioned into 24 separate regions: six from caudal to cephalic and 4 from dorsal to ventral. Biochemical assays were used to determine the quantities, relative to wet weight, of the major constituents of cartilage: chondrocytes, collagen and sulfated glycosaminoglycan.

Results—On average, each milligram of wet cartilage contained 24,900 cells, 73.9 micrograms collagen, and 17.1 micrograms sulfated glycosaminoglycan. Cell number showed no significant variation across the septa. In contrast, the caudal regions of the septa were associated with higher levels of collagen, the ventral regions correlated with higher levels of sulfated glycosaminoglycan, and the dorsal regions were associated with an elevated ratio of collagen to sulfated glycosaminoglycan.

Conclusion—This study represents the first characterization of the biochemical composition of native human septal cartilage across whole septa. Quantities of collagen and sulfated glycosaminoglycan showed region-specific variation across the septum. The localized pattern of collagen and sulfated glycosaminoglycan deposition are consistent with the significance of preserving the “L-strut” during rhinoplasty and other nasal reconstructive procedures. In addition, it may assist in defining design goals for tissue-engineered septal neocartilage constructs to meet specific reconstructive needs in the future.

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Level of Evidence—N/A**Keywords**

human septal cartilage; cartilage tissue engineering; collagen; glycosaminoglycan; chondrocyte

Introduction

Reconstructive surgery of the craniofacial skeleton often requires structural materials to repair cartilaginous defects created by trauma, congenital malformations or previous surgical resection. Autologous, allogenic and synthetic implants and grafts have been used in the past.^{1–3} Unfortunately, allogenic materials carry risks of immune rejection, disease transmission, and resorption, while synthetic materials are associated with risks of infection, extrusion, and foreign body reactions.³ Autologous cartilage tends to be the preferred material, with donor sites from the nasal septum, auricular conchal bowl, and the costal region. Of these materials, autologous nasal septal cartilage is preferred for nasal reconstruction due to its favorable mechanical properties, ease of harvest, and minimal donor site morbidity.^{3–5} However, the use of autologous nasal septal cartilage is limited by the finite amount of available tissue and suboptimal geometric structure for reconstruction of some defects.

These limitations have driven investigators to explore alternatives to cartilage autografts for reconstructive material. Tissue-engineered autologous septal neocartilage in the desired shapes and quantities can be generated by first amplifying chondrocyte numbers in culture, followed by differentiation in a three-dimensional culture system to restore the production of functional cartilaginous extracellular matrix (ECM) and finally a period of maturation and shaping to produce neocartilage constructs with the desired shape and structural properties for reconstruction.⁶

Adult cartilage contains chondrocytes plus an extensive ECM consisting of components that are synthesized and secreted by chondrocytes.⁷ Collagen is the most abundant molecular component by weight.⁸ Collagen fibrils in the ECM have been shown to provide the cartilage tissue with integrity, tensile stiffness, tensile strength, and resistance to swelling.⁹

Proteoglycan is the other main molecular component of cartilage, primarily in the form of aggrecan.¹⁰ Macromolecular aggrecan consists of a protein core attached to many sulfated glycosaminoglycan (sGAG) chains. The sGAG side chains in cartilage are responsible for its net negative charge that attracts positive ions and water into the ECM.¹¹ The mechanical properties of cartilage are due to the tendency of proteoglycans to swell with fluid, as balanced by the restraining function of the collagen.¹²

In a previous study in our laboratory, the density of cells and major matrix components in human nasal septal cartilage were determined in cartilage taken from the inferior strip of septum (just superior to the maxillary crest).¹³ Portions of this region of the septal cartilage are routinely harvested during septoplasty and septorhinoplasty procedures and thus represents a desirable source of starting material for septal cartilage tissue engineering. The current study extends this work by mapping densities of cells and ECM components across entire septa in order to better understand the relationship of cartilage composition with structural areas of the septum and to facilitate the design of tissue engineered septal cartilage for reconstruction.

Methods

Sample Preparation

The nasal septa from seven anonymous fresh-frozen cadavers were obtained with approval from the University of California, San Diego, Human Research Protections Program through the University of California, San Diego, School of Medicine. These specimens were frozen at -80°C in PBS containing proteinase inhibitors and thawed a day before sectioning. After thawing, the perichondrium was meticulously removed from the septum. The septum was then partitioned into 24 approximately equal segments, 6 from caudal to cephalic and 4 from dorsal to ventral, to create a grid. Samples from each segment were weighed to allow for determining cartilage component density, normalizing to wet weight.

Biochemical Testing

The samples to be used for biochemical assays were digested with Proteinase K (PK) in phosphate-buffered EDTA (0.5 mg/mL) overnight at 55°C . Cell density of the samples was tested using the PicoGreen DNA content determination assay (Invitrogen, Carlsbad, CA) adapted for human cartilage.¹⁴ Portions of each sample digest were mixed with PicoGreen reagent. Fluorescence was measured with an excitation light wavelength of 480nm and emission wavelength of 520nm in a spectrofluorimetric plate reader. Fluorescence values were converted to DNA quantity using standards of human DNA in the appropriate buffer solution. DNA content was normalized to determine cell number (7.8 picograms DNA/chondrocyte) and to milligram of wet tissue weight.

The sGAG content was determined using portions of the PK digests and the dimethyl-methylene blue (DMMB) reaction.¹⁵ Portions of each sample digest were mixed with DMMB dye. Absorbance at 525nm was measured in a spectrophotometric plate reader and compared to a plot of standards made from shark chondroitin sulfate type C (Sigma, St. Louis, MO). The majority of sGAG present in human cartilage is chondroitin sulfate with hyaluronan and keratan sulfate comprising smaller portions.¹⁶ Thus, use of chondroitin sulfate as a standard source for comparison is reasonable. Furthermore, determination of sGAG using this assay is highly correlated with fixed charge density in human cartilage.¹⁷ GAG content was normalized per milligram of wet tissue weight (before digestion) and to DNA content.

The hydroxyproline assay was used to determine the amount of total collagen in the PK digests.¹⁸ In a fume hood, a volume of 12.1 N hydrochloric acid (HCl) (Sigma, St. Louis, MO) equal to the sample volume was added to the sample and incubated at 110°C for 16–18 hours. The samples were removed from heat, allowed to equilibrate to room temperature, centrifuged briefly, and evaporated at 110°C (16–24 hours). The dried sample was dissolved in citrate assay buffer. Portions of the reconstituted samples and Hydroxyproline standards were reacted with Chloramine T reagent, and then p-Diaminobenzaldehyde, and the absorbance was read at 560 nm using an EMAX precision microplate reader (Molecular Devices, Sunnyvale, CA). The sample absorbance was converted to hydroxyproline content based on the Hydroxyproline standards. Hydroxyproline content was normalized per milligram of wet tissue weight (prior to digestion) and to DNA content. Hydroxyproline content was converted to collagen content using a mass ratio of collagen to hydroxyproline of 7.1.¹⁹

Histochemistry

Histochemistry was used to localize GAG and to evaluate tissue structure using Alcian Blue and Hemotoxylin and Eosin (H&E) staining solutions. Samples to be used for histochemistry were placed in Optimal Cutting Temperature (OCT) Compound and frozen.

They were sectioned in a cryostat at 10µm thickness. For histochemical localization of GAG, slides from each sample group were stained with 0.1% Alcian Blue in buffer (0.4 M magnesium chloride, 0.025 M sodium acetate, 2.5% glutaraldehyde, (pH 5.6) overnight, and destained with 3% acetic acid until clear. H&E staining was performed as previously reported.²⁰ Samples were observed using light microscopy and documented by photomicroscopy.

Statistical Analysis

Analysis was performed using Systat 10.2 (Systat Software, Chicago, IL). Differences in cells, sGAG, collagen, and ratio of collagen to sGAG in each segment were assessed using one-way analysis of variance (ANOVA). If the ANOVA identified an overall significant effect, post-hoc Tukey's HSD tests were used to identify significant differences between segments. Linear regression analysis was used to analyze the relationship between septal region and collagen, sGAG and ratio of collagen to sGAG. A difference was considered significant when $p < 0.05$.

Results

Whole septa from 7 cadavers were divided into 24 approximately equal segments to create a grid with six divisions from caudal to cephalic (CD1, CD2, MCD, MCP, CP1 and CP2) and 4 divisions from dorsal to ventral (D, MD, MV, V) (Figure 1). Each cartilage segment was assayed for number of cells, micrograms (µgs) total collagen and µgs sGAG per milligram wet weight. The mean quantities of septal cartilage components for each segment are presented in Tables 1 and 2.

Wet cartilage segments were found on average to contain 24,900 cells per milligram (range, 17,900–33,000). ANOVA and linear regression analyses confirmed that the cellularity did not vary significantly across the segments (caudal to cephalic, $p = 0.659$ and $p = 0.156$, dorsal to ventral $p = 0.636$ and $p = 0.348$ respectively, Tables 1 and 2). In addition, H&E staining of cartilage segments showed comparable overall structure and cellularity (data not shown).

Cartilage segments contained an average of 73.9 µg collagen per milligram wet weight (range, 64.5–86.4 µg). The amount of collagen per milligram was not significantly different from dorsal to ventral segments by ANOVA ($p = 0.696$). Linear regression analysis demonstrated a significant decrease in collagen levels from caudal to cephalic segments ($p = 0.007$, data not shown). This result was confirmed by ANOVA ($p = 0.003$, Table 1).

The average amount of sGAG in cartilage segments was 17.1 µg per milligram wet weight (range, 12.2–23.8 µg). Amounts of sGAG did not vary from caudal to cephalic segments when analyzed by linear regression ($p = 0.516$). However, average amounts of sGAG increased from dorsal to ventral segments when analyzed by either ANOVA or linear regression ($p < 0.005$, Figure 2A). This finding was corroborated by Alcian Blue staining of dorsal and ventral segments (Figure 3).

The average ratio of collagen to sGAG was 4.4 (range, 3.2–6.2) with higher ratios in the dorsal segments when compared with ventral segments by linear regression and ANOVA analyses ($p < 0.005$, Figure 2B).

Discussion

The results described here indicate that the major ECM components of human septal cartilage are not uniformly distributed throughout the septa. Collagen content was positively correlated with caudal septal segments while increased sGAG levels were correlated with

ventral segments. This finding was supported by histochemical staining with Alcian Blue. Furthermore, the ratio of collagen to sGAG was positively associated with dorsal septal segments. In contrast, the concentration of cells was not significantly different across the septa. On average each milligram of cartilage contained 24,900 cells, 73.9 µg collagen, and 17.1 µg sGAG with an average collagen to sGAG ratio of 4.4. To our knowledge no previous studies have quantified the densities of cells and major matrix components across whole human septa.

In an earlier report, we analyzed the cellularity and major matrix components of human nasal septal cartilage harvested from the ventral septum (just superior to the maxillary crest) during routine septoplasty and septorhinoplasty procedures. In that study, mean values per mg cartilage wet weight were 24,900 cells (range, 3,700–51,800), 77.4µg collagen (range, 45.8–128.6 µg) and 28.7 µg sGAG (range, 13.6–44.6µg) with a mean collagen to sGAG ratio of 3.03.¹³ This is comparable to the values found in the current study, particularly of the ventral region in the septum

The composition of cartilage in a number of tissues varies depending on its specific location. For example, articular cartilage composition varies depending on joint location, region within the joint and depth from the joint surface. The reported cell density of human articular cartilage is about 2-fold lower than the value measured in this study.^{21,22} Relative amounts of septal cartilage ECM components measured in this study were lower than reported human femoral condyle values of 150–200 µg collagen and 30–60 µg sGAG per milligram wet weight.²³ Although both articular and septal cartilage are categorized as hyaline cartilage, they have different embryonic origins and inherent function that may explain some of their compositional differences.²⁴

The mechanical stiffness, strength, and stability of cartilage are influenced by the relative abundance of certain structural molecules in the ECM of the chondrocytes.^{25,26,27} In human articular cartilage sGAG molecules endow the tissue with a fixed negative charge that increases the tendency of the tissue to swell and resist compressive loading,²⁸ while the tensile strength has been attributed primarily to the collagen network.^{25–30} Within articular cartilage, the collagen content is approximately 2–10 times higher than GAG content depending on the region of the tissue sampled and the mechanical load that the tissue experiences, with averages ranging from 3.1 to 4.9.²⁷ Mechanical strength and stiffness strongly correlate with increased ratios of collagen to GAG.²⁷ In this study of native human nasal septum, the average collagen content was found to be approximately 4.4 times higher than GAG content with a significantly higher ratio in the dorsal region of the nasal septum. Based on the aforementioned studies conducted in articular cartilage, it is expected that the dorsal region of the septum would also have the greatest mechanical stiffness, strength and stability.

It is heavily emphasized in rhinoplasty and nasal reconstructive surgery literature that the dorsal and caudal septum “L-strut” provide structural stability to the nose as part of the critical regions for nasal support.^{31–34} Deformities or deficiencies in this region can create aesthetic and functional problems such as saddle nose deformity, malpositioned tip, twisted nose, and internal valve insufficiency; therefore, elevated collagen in the caudal regions as well as increased collagen to sGAG ratios in the dorsal regions of the nasal septum as reported in this study support the hypothesis that this region would also provide greater stability and support, and tends to scientifically substantiate the value of the L-strut preservation.

Conclusion

This study provides a quantitative map of human nasal septal composition across whole human septa. In all probability, tissue engineered septal cartilage will have a role in future reconstructive surgery of the head and neck. If it is to endure and adjust to the mechanical demands of the in vivo environment, tissue engineered septal cartilage tissue must resemble native tissue in its morphologic, histologic, biochemical and biomechanical properties. Therefore, a thorough understanding of native septal cartilage composition across the human septum will serve as a baseline for designing engineered neocartilage that meets specific reconstructive goals. Future experimental work will include mapping the mechanical properties of cartilage across whole human septa.

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Figure 1.
Photo of human cadaveric septa illustrating the 24 divisions from caudal to cephalic (CD1, CD2, MCD, MCP, CP1, CP2) and from dorsal to ventral (D, MD, MV, V).

Figure 2.

Sulfated glycosaminoglycan (sGAG) content (A) and the ratio of collagen to sGAG (B) as a function of septal region (dorsal (D), middle dorsal (MD), middle ventral (MV), and ventral (V)). Linear regression analysis demonstrates a positive trend in sGAG abundance toward the ventral septum as well as a positive association of the collagen to sGAG ratio toward the dorsal septum ($p<0.005$).

Figure 3. Alcian Blue staining of a representative dorsal (A) and ventral (B) segment from one subject indicates increased accumulation of sGAG in the ventral section of the septa (10x magnification).

Table 1

Caudal to Cephalic Variation in Septal Cartilage Components *

Segment	cells, per mg WW	collagen, $\mu\text{g}/\text{mg}$ WW	sGAG, $\mu\text{g}/\text{mg}$ WW	collagen:sGAG
Overall	24,900 $\pm$ 3,910	73.9 $\pm$ 6.41	17.1 $\pm$ 3.02	4.44 $\pm$ 0.79
Caudal 1	25,800 $\pm$ 3,150	82.9 $\pm$ 3.41	17.7 $\pm$ 2.58	4.75 $\pm$ 0.54
Caudal 2	26,500 $\pm$ 2,600	76.7 $\pm$ 5.55	17.2 $\pm$ 2.06	4.50 $\pm$ 0.58
Middle Caudal	27,200 $\pm$ 3,900	72.6 $\pm$ 3.68	16.0 $\pm$ 2.24	4.62 $\pm$ 0.81
Middle Cephalic	25,200 $\pm$ 6,230	68.5 $\pm$ 5.19	18.2 $\pm$ 4.46	3.89 $\pm$ 0.70
Cephalic 1	22,000 $\pm$ 2,450	79.4 $\pm$ 7.61	18.3 $\pm$ 4.16	4.22 $\pm$ 1.07
Cephalic 2	23,000 $\pm$ 3,640	73.7 $\pm$ 7.84	15.3 $\pm$ 2.66	4.65 $\pm$ 1.10
<i>P</i> value **	0.659	0.003	0.443	0.558
<i>P</i> value ***	0.156	0.007	0.516	0.700

Abbreviations: WW, cartilage wet weight; sGAG, sulfated glycosaminoglycan

*
Data are presented as mean $\pm$ SD.*P* values are based on analysis of variance (**) or linear regression (***) analyses between segments.

Table 2Dorsal to Ventral Variation in Septal Cartilage Components^{*}

Segment	cells, per mg WW	collagen, $\mu\text{g}/\text{mg}$ WW	sGAG, $\mu\text{g}/\text{mg}$ WW	collagen:sGAG
Overall	24,900 $\pm$ 3,910	73.9 $\pm$ 6.41	17.1 $\pm$ 3.02	4.44 $\pm$ 0.79
Dorsal	22,000 $\pm$ 4,140	74.0 $\pm$ 5.13	13.7 $\pm$ 0.90	5.43 $\pm$ 0.59
Middle Dorsal	25,200 $\pm$ 3,180	72.1 $\pm$ 7.31	15.8 $\pm$ 1.32	4.55 $\pm$ 0.14
Middle Ventral	23,800 $\pm$ 3,340	78.3 $\pm$ 8.72	19.1 $\pm$ 2.17	4.04 $\pm$ 0.59
Ventral	28,000 $\pm$ 4,680	75.1 $\pm$ 6.49	19.8 $\pm$ 2.30	3.73 $\pm$ 0.44
<i>P</i> value ^{**}	0.636	0.696	< 0.001	< 0.001
<i>P</i> value ^{***}	0.348	0.880	< 0.001	< 0.001

Abbreviations: WW, cartilage wet weight; sGAG, sulfated glycosaminoglycan

^{*} Data are presented as mean $\pm$ SD.*P* values are based on analysis of variance (**) or linear regression (***) analyses between segments.