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Human Nasal Cartilage Ultrastructure: Characteristics and Comparison Using Scanning Electron Microscopy

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Abstract

Objectives/Hypothesis—Human nasal cartilage is hyaline cartilage, although the function and loads placed on it are different depending on the location. We hypothesized that important differences exist between the nasal septal cartilage and lower lateral cartilage (LLC) ultrastructures. Such differences would be important in the field of cartilage engineering.

Methods—Ten specimens (6 septum and 4 LLC) of cartilage from patients undergoing nasal surgery (rhinoplasty or septoplasty) were obtained and examined using scanning electron microscopy. Micrographs were then analyzed and measured using photograph analysis software.

Results—The collagen fibers of septal cartilage were found to be arranged in a mesh framework, with larger lacunae and thicker fibers measuring 3.18 μm (standard deviation = 0.75 μm), with a 99.9% confidence interval of 2.74 to 3.54 μm . LLC fibers, on the other hand, were arranged in less-organized sheets, with fibrous extensions, and had fewer, narrower lacunae. The fibers from the LLC averaged 2.29 μm , with a 99.9% confidence interval of 1.17 to 3.42 μm .

Conclusions—Significant ultrastructural differences exist between the cartilage of the nasal septum and LLC. These are almost certainly the result of their embryologic origins and different forces placed on the structures they support. A less-organized pattern with smaller collagen fibers is present in the LLC versus the more-organized, layered, thicker collagen fibers of the septum. These differences may prove to be critical in the future of cartilage engineering.

Keywords

Scanning electron microscopy; cartilage; nasal; tissue engineering

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INTRODUCTION

Tissue engineering is a rapidly developing area of research. The goal of this work is the development of three-dimensional tissue constructs in an in vitro or ex vivo environment to form a clinically functional tissue. Presumably, this tissue would possess histologic, biochemical, and biomechanical properties identical to the native, healthy tissue it is intended to replace. One particular area of development has been that of tissue-engineered cartilage.¹⁻⁶

Recently, absorbable matrices have been constructed as a scaffold for chondrocytes to adhere to and form cartilage.⁷⁻⁹ Some success has been achieved in this area; however, adaptations to human models are currently focused largely on orthopedic problems. Fortunately, this work is paving the way for progress in developing suitable replacements for nasal cartilage.

Although much microscopic imaging of the cartilages of the body has been performed, no ultrastructural analyses could be found comparing the different cartilages of the nose such as that of the septum with that of the lower lateral cartilage (LLC). It is our hypothesis that because the roles of these two important cartilaginous structures differ, their ultrastructural makeup will as well. This could prove to be important information for future scaffold development as it relates to engineered nasal cartilage.

MATERIALS AND METHODS

Discarded cartilage from 10 healthy patients undergoing septoplasty or rhinoplasty was obtained from our university hospital. Specimens were immediately placed into 10% phosphate-buffered formalin and labeled with the source (septum or ala) of the cartilage and some basic nonidentifying information about the patient from whom they were obtained. Specimens were fixed in this solution for 24 hours and then transferred to phosphate-buffered solution. LLC specimens were pinned to a piece of cork because of their natural curvature and to prevent further deformation during dehydration. Septal cartilage specimens were not pinned and did not significantly distort after dehydration. Specimens were postfixated with 1% aqueous osmium tetroxide and then dehydrated with progressive concentrations of ethanol-water, followed by progressive concentrations of ethanol-hexamethyldisilazane. The samples were mounted on sample stubs, gold coated using a PAC-1 Pelco Advanced Coater (Pelco International, Redding, CA), and imaged with a Philips 515 scanning electron microscope (FEI Co., formerly Philips Optics, Hillsboro, OR).

The resultant images were then reviewed and analyzed using photograph editing software (Adobe Photoshop 7.0; Adobe Systems, Inc., San Jose, CA). Fiber width measurements were taken from all specimens (where present) from each micrograph at $\times 3,000$ magnification and analyzed using spreadsheet software (Microsoft Excel; Microsoft Corporation, Redmond, WA).

RESULTS

The scanning electron micrographs yielded interesting general observations as well as numeric data. It was first noted that the LLC specimens demonstrated a looser arrangement of collagen fibers, as is notable in Figures 1 and 2. One can observe that there is a more heterogeneous mixture of fiber thickness and that the arrangement tends to be less ordered with narrowed lacunae and thin collagen sheets. In addition, a large number of fine fibrous projections appear to be randomly associated with the larger collagen fibrils. Conversely, the septal cartilage images in Figures 3 and 4 demonstrate thick sheets of broad, highly organized collagen fibers. These contain larger lacunae (which also appear more abundant) and little or no fibrous projections.

Measurements taken from all specimens at the highest resolution ($\times 3,000$) indicate a difference in fiber width. For the septal cartilage specimens, the average fiber width measured $3.18\text{ }\mu\text{m}$, with a 99.9% confidence interval of 2.74 to $3.54\text{ }\mu\text{m}$. LLC fibers averaged $2.29\text{ }\mu\text{m}$, with a 99.9% confidence interval of 1.17 to $3.42\text{ }\mu\text{m}$.

DISCUSSION

The nasal septum serves as major structural support for the nasal dorsum, is a shock absorber of sorts for trauma, divides the two nasal cavities (improving laminar flow when straight), and is important in the growth and development of its surrounding structures. Such a structure requires a more rigid character while still having the viscoelastic properties of cartilage that resist fracture and conform to daily activities. The LLCs, on the other hand, serve to support the nasal tip, prevent external nasal valve collapse, and give cosmetic definition to the nose. The nasal tip is highly varied and much softer than the septum. It is therefore not surprising that septal cartilage would have a more robust, organized structure than the LLC.

Perhaps more important is the fact that these structural differences may be important in cartilage engineering as it pertains to nasal reconstruction. As scaffolds are developing with greater sophistication, it may be possible to tailor the appropriate combination for total nasal reconstruction. In the recent work published by Griffon et al.,⁷ scanning electron micrographs of their scaffoldings along with early chondrocyte adherence bear some interesting resemblance to that of septal cartilage.

It is notable in this study that a limited number of LLC and septal cartilage specimens were studied. Notwithstanding this, the few specimens we studied showed very little variability in the differences we have described and demonstrated in photomicrographs. Conceivably, further differences exist in the nasal cartilage of patients with prior septal surgery/injury, significant septal deviation, systemic disease, chronic medication exposure, or at the extremes of age.

CONCLUSIONS

Significant ultrastructural differences exist between the cartilage of the nasal septum and LLC. These are almost certainly a result of the embryologic origins and different forces

placed on the structures they support. A less-organized pattern with smaller collagen fibers is present in the LLC versus the more-organized, layered, thicker collagen fibers of the septum. These differences deserve attention as cartilage engineering advances.

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Fig. 1. Scanning electron micrographs of human lower lateral cartilage. Each successive image from A to C is of increasing magnification of $\times 1,000$, $\times 2,000$, and $\times 3,000$. (A) General arrangement of superficial structures. (B and C) Higher magnifications show thin collagen fibers clearly arranged in loosely woven pattern around narrower lacunae.

Fig. 2.

Scanning electron micrographs of different human lower lateral cartilage specimen than those in Figure 1. Each successive image from A to C is of increasing magnification of $\times 1,000$, $\times 2,000$, and $\times 3,000$. (A) General arrangement of superficial structures. This specimen demonstrates increased number of fine, fibrous projections compared with Figure 1. (B and C) Higher magnifications show thin collagen fibers under fibrous projections and at least one chondrocyte.

Fig. 3. Scanning electron micrographs of human nasal septal cartilage. Each successive image from A to C is of increasing magnification of $\times 1,000$, $\times 2,000$, and $\times 3,000$. (A) General arrangement of tightly associated sheets of collagen fibers. (B and C) Higher magnifications show thick collagen fibers clearly arranged in tight linear array around broader, more round lacunae.

Fig. 4. Scanning electron micrographs of different human nasal septal cartilage than that in Figure 3. Each successive image from A to C is of increasing magnification of $\times 1,000$, $\times 2,000$, and $\times 3,000$. (A) Arrangement of tightly associated sheets of collagen fibers. (B and C) Higher magnifications show more of same thick collagen fibers and round lacunae as noted in Figure 3.