Histological structure of the nasal cartilages and their perichondrial envelope II. The perichondrial envelope of the septal and lobular cartilage*

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SUMMARY The perichondrial envelopes of the septal cartilage and the lateral crus of the lobular cartilage were studied in serial coronal sections of five human noses. To differentiate between the various tissue components, the sections were stained according to Mallory-Cason, Azan, Herovici, Verhoeff-van Gieson, and Lawson. Collagen types I and II were immunohistochemically stained. The results demonstrated that the perichondrium of the septal cartilage and the lateral crus of the lobular cartilage consists of a homogeneous layer of type I collagen fibers and elastic fibers. The elastic fibers have a network-like arrangement and are most numerous in the perichondrium of the lateral crus of the lobular cartilage. Clearly distinguishable zones in the perichondrial envelopes could not be observed. The perichondrium on the outside of the lateral crus of the lobular cartilage and the triangular cartilage is significantly thicker than the inner perichondrium. It is speculated that these morphological characteristics of the perichondrial envelopes are related to functional differences between the cartilages. The mobility of the lateral crus of the lobular cartilage requires a higher content of elastic fibers in its perichondrium than the more rigid septal cartilage. A thicker outer perichondrium of the lateral crus of the lobular cartilage and the triangular cartilage may be related to muscular forces that are exerted on the outer side of the cartilages only.

INTRODUCTION

The perichondrium is a dense connective tissue layer that covers mammalian cartilage except at articular surfaces, where cartilage is exposed to synovial fluid. Recognizing that the perichondrial structure varies with cartilage location, it is suggested that the variation is related to differences in functional requirements. Nevertheless, a general structural plan in which three zones may be distinguished has been proposed: 1. a loose outer layer containing blood vessels and nerve fibers; 2. a main layer of dense connective tissue; and 3. an internal zone of fusion with the extracellular matrix (ECM) of the underlying cartilage ⁽¹⁾. The internal zone is not recognized as a separate layer by all authors ⁽²⁾.

The perichondrium consists of cellular elements – the perichondrocytes, which are fibroblasts – and the ECM. Collagen and elastic fibers are found in the ECM, which is synthesized by perichondrocytes. The quantity and orientation of the fibrous elements varies according to the cartilage type and site ⁽¹⁾. Detailed descriptions of the perichondrium of particular nasal cartilages are still lacking, however. Considering the functional differences between septal and lobular cartilages, as discussed in the accompanying article by Popko et al., ⁽³⁾ the corresponding perichondria may demonstrate variations in morphology. The present study examines the morphological details of the perichondrium of human septolateral and lobular cartilages.

MATERIAL AND METHODS

Staining of specimens

Five human noses from cadavers of Caucasian origin aged 68, 75, 75, 80, and 87 were studied.

The specimens were preserved, fixed, and sectioned as described in the accompanying article ⁽³⁾. Several staining methods were applied. A modified Mallory-Cason trichrome stain ⁽⁴⁾ was used to discriminate between bone, cartilage, and connective tissue. Azan stain was applied to visualize collagen fibers, Herovici stain to discriminate between young and mature collagen. Verhoeff-van Gieson stain was used to demonstrate elastic fibers. Type I and type II collagens were immunohistochemically demonstrated as described in the accompanying article ⁽³⁾.



Figure 1. Septal perichondrium (Mallory-Cason staining). left) The existence of a dense inner and a loose outer layer is suggested but is probably due to a processing artifact. right) The perichondrium is a homogeneous structure without clearly distinguishable zones.

Table 1. Thickness (mean \pm SD in μ m) of the outer and inner perichondrium of the lateral crus of the right and left lobular cartilages and triangular cartilages.

	Lateral crus of lobular cartilage		Triangular cartilage	
	right side	left side	right side	left side
	(n=13)	(n=14)	(n=7)	(n=4)
outer perichondrium inner perichondrium	49.0 ± 11.1	47.5 ± 13.2	53.3 ± 10.4	46.5 ± 13.0
	42.0 ± 10.4	37.9 ± 11.1	40.4 ± 17.3	36.0 ± 12.3

Measurements

In the Mallory-Cason stained sections, the thickness of the perichondrium of the triangular and the lateral crus of the lobular cartilages was measured with the aid of a graticule in the eyepiece of a microscope. Measurements were made on the inside and the outside of the cartilages. On every side of a cartilage, three parts of the perichondrium were measured at random. The mean values were used for statistical analysis. It was not possible to make truly random measurements on all sections. In case of artifacts, the part of the perichondrium closest to the artifact was measured. If there was any doubt about the validity of the measurements, the data were not included in the statistical analysis.

Statistics

The data were analyzed by paired t tests (one-tailed).

RESULTS

Septal perichondrium

Several of the Mallory-Cason stained sections suggest the existence of a dense inner layer and a loose outer layer in the septal perichondrium. In the same sections, however, parts of the perichondrium appear as a single layer of dense collagen bundles (Figure 1). In some specimens, the nose had not been processed as a whole unit but as individual septal and lobular cartilages instead. Especially in these specimens, it was difficult to distinguish between a dense inner and a loose outer layer. Therefore, the artifacts might suggest the existence of a loose outer layer.

The septal perichondrium is made up of a homogeneous dense layer containing collagen and elastic fibers (Figures 2 and 3). Its thickness is approximately 150-200 μ m. The collagen is of type I (Figures 3 and 4). Type II collagen is not present in the perichondrium. The transition from cartilage to the perichondrium is not very well defined. Elastic fibers are not abundant, as demonstrated by Verhoeff-van Gieson stain (Figure 2).

Lobular and triangular cartilage perichondrium

The perichondrium of the lateral crus of the lobular cartilage consists of a homogeneous layer of collagen and elastic fibers (Figure 5). It is thinner than the septal perichondrium. However, in the lateral crus of the lobular cartilage, the outer perichondrium is distinctly thicker than the inner one. The perichondrium of the triangular cartilage gives a similar impression. Evidence was obtained by thickness measurements. The results demonstrate that the outer perichondrium



Figure 2. Septal perichondrium (Verhoeff-van Gieson staining). There are a few black-stained elastine fibers running from the perichondrium to the cartilage.



Figure 3. Septal perichondrium (type I collagen staining). There is an abundance of collagen type I in the perichondrium.



Figure 4. Septal perichondrium (type II collagen staining). There is no type II collagen in the perichondrium.



Figure 5. left and right Perichondrium of lateral crus of lobular cartilage (Mallory-Cason staining). The outer perichondrium (top) is distinctly thicker than the inner perichondrium.

is thicker than the inner perichondrium, both in the lateral crus of the lobular cartilage and the triangular cartilage (Table 1). The differences between the means are statistically significant for right and left lobular and triangular cartilages (p < 0.05). The differences between right and left sides are not statistically significant.

Both the Verhoeff-van Gieson and the Lawson staining of the perichondrium of the lobular cartilage demonstrate an abundance of elastic fibers (Figures 6 and 7). The number of elastic fibers clearly exceeds that of the septal cartilage. The numerous elastic fibers run either parallel to the surface of the cartilage or in oblique or perpendicular directions.

Immunohistochemistry demonstrates that the collagen is of type I and that type II is not present (Figures 8 and 9).

DISCUSSION

The main results of this histological study of the perichondrial envelope of the septal and lobular cartilages are the following: 1. the absence of clearly distinguishable zones in the perichondrium; 2. a significant difference in thickness between outer and inner perichondrium of the triangular and lateral crus of the lobular cartilage; and 3. a higher number of elastic fibers in the perichondrium of the lobular cartilage in comparison with that of the septal cartilage.

A major study by Bairati et al. on the structure of the perichondrium of various cartilages in animals and humans indicated the presence of three zones: 1. a loose outer layer containing blood vessels; 2. a main layer of dense connective tissue; and 3. an internal zone of fusion with the ECM of the underlying cartilage⁽¹⁾. The authors included human nasal cartilages in their material, but the exact origin of these cartilages - lobular, triangular, or septal - is not clear. They concluded that in "nasal cartilages" the perichondrium is tightly attached to the underlying cartilage and that the border between the internal zone and the ECM of the cartilage is not very well defined, in contrast to ear cartilage. Our results are in line with this description; in many sections, it was hard to define the transition. This finding adds to the fact that in the nose the perichondrium shares a biomechanical role with the cartilage and that cartilage and perichondrium should be viewed as a functional unit. Our findings are not consistent with the sup-



Figure 6. Perichondrium of lateral crus of lobular cartilage (Verhoeffvan Gieson staining). There is an abundance of black-stained elastine fibers that run parallel and perpendicular to the cartilaginous surface. Some fibers run in an oblique direction.



Figure 7. Perichondrium of lateral crus of lobular cartilage (Lawson staining). Many elastine fibers in the perichondrium run in various directions and form a network.



Figure 8. Perichondrium of lateral crus of lobular cartilage (type I collagen staining). There is an abundance of collagen type I in the perichondrium.

posed presence of a loose outer layer, however. In those sections where a loose outer layer was observed, it was highly likely that this was an artifact due to tissue fixation and sectioning. In many other sections, the perichondrium was seen as a homogeneous layer of collagen and elastic fibers. We therefore conclude that in both septal and lobular cartilages, the perichondrium does not have clearly distinguishable zones but is a homogeneous structure instead.

We speculate that differences in thickness of the outer and inner perichondrium in the lobular and triangular cartilages are related to the forces that are applied to the outer and inner sides of these cartilages. Muscles are found on the outside of the cartilages only. The dilatator naris muscle attaches to the lateral crus of the lobular cartilage. The transverse part of the nasalis muscle does not attach to the triangular cartilage but overlies it ⁽⁵⁾. It moves the nasal skin; therefore, gliding movements between this muscle and the triangular cartilage should be permitted.

In our view, the finding of numerous elastic fibers in the perichondrium of the lateral crus of the lobular cartilage can be explained by its mobility. The posterior part of the lateral crus shows an in- and outward bending during respiration ⁽⁵⁾. In contrast, the septal cartilage is more rigid, which is in line with its support function as discussed in the accompanying article ⁽³⁾. It therefore needs fewer elastic fibers in its enveloping perichondrium. The direction of elastic fibers in the perichondrium is parallel to the surface of the cartilage but also oblique and perpendicular. This network-like arrangement helps maintain the complex shape of the lobular cartilage.

In conclusion, the outer and inner perichondrial envelopes of the septal, lobular, and triangular cartilages each have their own morphological characteristics that are related to functional differences between the cartilages.



Figure 9. Perichondrium of lateral crus of lobular cartilage (type II collagen staining). There is no type II collagen in the perichondrium.

REFERENCES

- Bairati A, Comazzi M, Gioria M. A comparative study of perichondrial tissue in mammalian cartilages. Tissue & Cell 1996; 28: 455-468.
- 2. Huizing EH, de Groot JAM. Functional Reconstructive Nasal Surgery. Thieme, Stuttgart-New York 2003; pp 27-32.
- 3. Popko M, Bleys RLAW, De Groot JW, Huizing EH. Histological structure of the nasal cartilages and their perichondrial envelope I. The septal and lobular cartilage. Rhinology 2007; 45: 148-152.
- 4. Van Leeuwen MBM, Deddens AJH, Gerrits PO, Hillen B. A modified Mallory-Cason staining procedure for large cryosections. Stain Technol 1990; 65: 37-42.
- 5. Bruintjes TD, van Olphen AF, Hillen B, Huizing EH. A functional anatomic study of the relationship of the nasal cartilages and muscles to the nasal valve area. Laryngoscope 1998; 108: 1025-1032.
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