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

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SUMMARY

The cellular elements and extracellular matrix of the nasal septal cartilage and the lateral crus of the lobular cartilage were studied in serial coronal sections of five human cadaver noses. To discern the various tissue components, the sections were stained according to the methods of Mallory-Cason, Azan, Herovici, Verhoeff-van Gieson, and Lawson as well as by immunohistochemistry to demonstrate the presence of collagen type I and II.

A characteristic gradual transition of the chondrocytes was observed in both septal and lobular cartilage: from numerous small flat cells oriented parallel to the surface of the cartilage to less numerous larger ovaloid cells oriented perpendicular to the surface. This difference between the peripheral and central zones of the cartilage was particularly marked in lobular cartilage.

Both septal and lobular cartilage have a high density of type II collagen but almost none of type I. The peripheral zones of the matrix showed a higher density of collagen than the central zone. This difference was more pronounced in septal than lobular cartilage. The high density of type II collagen in septal cartilage, particularly in the peripheral zones, suggests that one of the primary tasks of the septum is providing stiffness to the external nose. That idea is consistent with findings from our study of the perichondrial envelope.

INTRODUCTION

The framework of the human nose is comprised of five main cartilaginous elements: the septal cartilage; two triangular cartilages (together constituting the septolateral cartilage); and two lobular cartilages. In addition, there are various small sesamoid cartilages in the intercartilaginous joint areas and two or three accessory cartilages in the lateral soft-tissue areas [1,2].

These cartilaginous structures are made up of hyaline cartilage, consisting of chondrocytes and an extracellular matrix (ECM). The chondrocytes have the capacity to synthesize the cartilage matrix. Some have short cilia extending into the matrix. There are no contacts between the individual cells. The matrix consists of water (80%) and a macromolecular framework of collagens, proteoglycans, and non-collagenous proteins, respectively amounting to 60, 25-35, and 15-20% of its dry weight. Multiple types of collagens are present, in particular types II (90-95%), IX, and XI, which form the cross-banded fibrils that provide stiffness [3,4]. In studies of articular cartilage, Buckwalter and Mankin distinguished four layers or zones with different morphological and functional features [3].

Cartilage has a low-level metabolism. Besides lacking vascular and nerve supply, it has no healing capacity, which has major implications for nasal pathology and surgery [5,6].

The nasal cartilaginous framework provides the external nasal pyramid with a degree of stability and mobility. The present study examines the morphology and arrangement of cells and the composition of the matrix in these cartilages.

MATERIAL AND METHODS

Staining of specimens

We studied five human noses from cadavers of Caucasian origin aged 68, 75, 75, 80, and 87 years.

The specimens were preserved and fixed in 4% buffered formaldehyde, decalcified with sodium formiat solution, and then dehydrated in increasing concentrations of alcohol and embedded in paraffin. Serial sections of 10, 20, and 25μm thickness were cut in the coronal plane at intervals of 200 and 400μm. They were mounted on glass slides and stained according to the following methods. A modified Mallory-Cason trichrome stain was used to discriminate between bone, cartilage, and connective tissue [7]. Azan stain was applied to visualize collagen fibers and Herovici stain to discriminate between young and mature collagen. Verhoeff-van Gieson stain was used to demonstrate elastic and collagen fibers, whereas Lawson stain was used to demonstrate the presence of elastic fibers.

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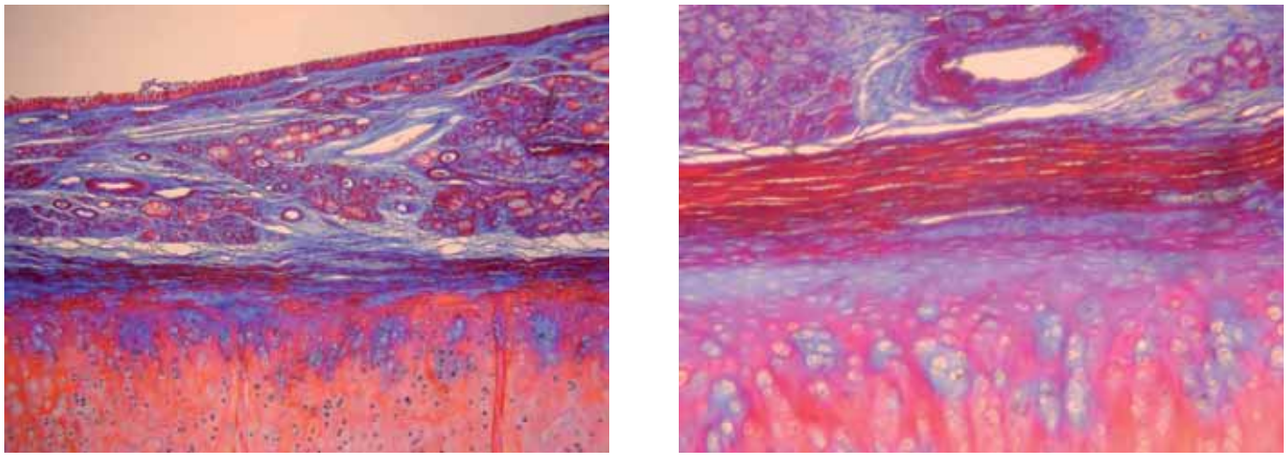

Figure 1. Septal cartilage with perichondrium and mucosa (Mallory-Cason staining). Left: overview, right: detail. In the peripheral zones of the cartilage the chondrocytes are numerous, small, flat, and oriented parallel to the surface of the cartilage. In the more central zones they are larger, more round, clustered, and oriented perpendicular to the surface. The bluish staining of the matrix in certain areas of the peripheral zones and around some of the chondrocytes indicates a rich collagen content.

Immunohistochemistry for types I and II collagen was performed by the ABC method at room temperature. The sections were washed in PBS and pretreated with 1% H_2O_2. They were washed again in PBS, followed by pre-incubation in 5% goat serum (type I) or 5% rabbit serum (type II) in PBS for 60 minutes. Subsequently, the sections were incubated overnight in anti-collagen I antibodies (Abcam plc., Cambridge, U.K.) or anti-collagen II antibodies (Chemicon International Inc., Temecula, CA, U.S.A.) diluted 1:400 or 1:100 respectively in PBS containing 2% bovine serum albumin (BSA). After washing in PBS, the specimens were incubated for 60 minutes in goat-anti-rabbit IgG biotin (type I) or rabbit-anti-mouse IgG biotin (type II) (Dako, Glostrup, Denmark) diluted 1:600 in PBS containing 2% BSA. The sections were washed in PBS and subsequently incubated for 30 minutes in ABC complex (Dako, Glostrup, Denmark) diluted 1:800 in PBS. The sections were washed in PBS and subsequently in 0.01 M sodium acetate. After incubation for 5 minutes in 0.04 % diaminobenzidine and 0.01% H_2O_2 in 0.01 M sodium acetate, the sections were washed in PBS and mounted in Entellan (Merck, Darmstadt, Germany).

RESULTS

Septal cartilage

Cellular elements: Chondrocytes, which are the only cellular elements in septal cartilage, are clearly demonstrated by the Mallory-Cason staining (Figure 1). They differ in size and shape but also in metabolic activity. Based on these differences, we can distinguish three zones and a gradual transition between them. In the peripheral zones, we find the young cells. They are numerous, small, flat, and oriented parallel to the surface of the cartilage.

Cells in the intermediate zones are less numerous and more ovaloid. Their axis runs more perpendicular to the cartilaginous surface.

The lowest density of chondrocytes is found in the central zone. Here, the cells are spheroidal and more or less aligned in columns perpendicular to the cartilaginous surface.

Extracellular matrix: The extracellular matrix of septal cartilage shows distinct differences in composition between the peripheral and central zones. The small and flat young cells in the peripheral zones are surrounded by a homogeneous light-blue staining material, suggesting a rich collagen content (Figure 1). In the intermediate zones, the matrix stains more pinkish. In the central zone, the chondrocytes are surrounded by more or less blue-staining material, whereas the remaining matrix stains pink to red.

With the Azan staining, the peripheral zones appear bluish, in contrast to the more central zones that stain more reddish (Figure 2). This confirms the presence of a higher density of collagen fibers in the outer areas.

Using Herovici staining, the matrix of the peripheral zones stains light blue, characteristic of young collagen, whereas the matrix of the central zones has a more reddish color, specific

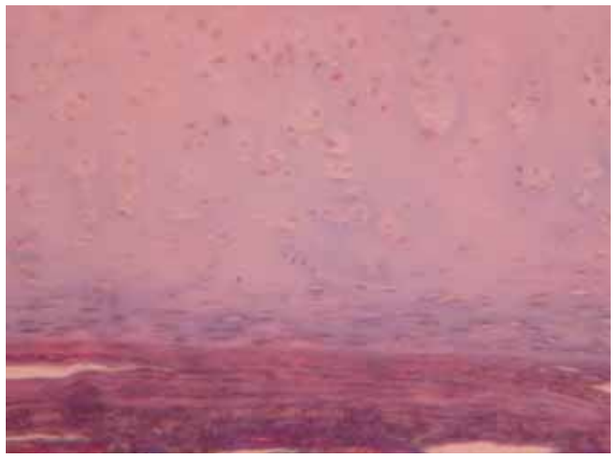

Figure 2. Septal cartilage with perichondrium (Azan staining). Bluish color of peripheral matrix indicates a high density of collagen fibers.

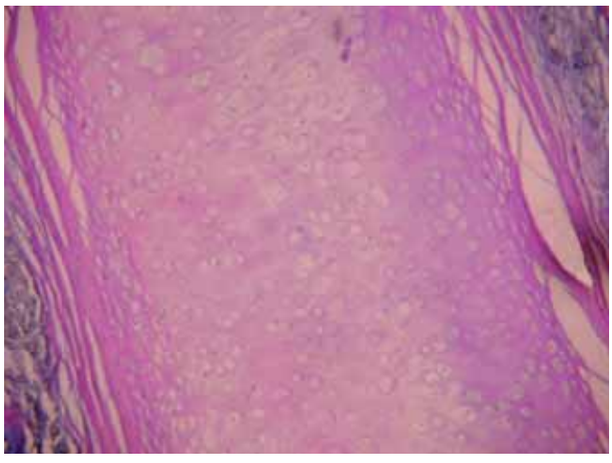

Figure 3. Septal cartilage with perichondrium (Herovici staining). Light-blue coloring of the matrix in the peripheral zones demonstrates the presence of young collagen.

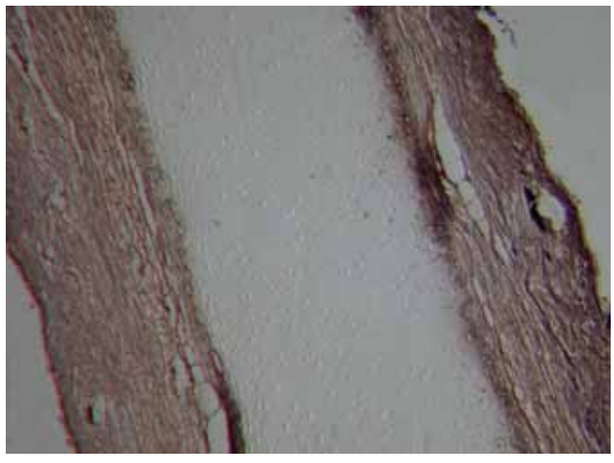

Figure 4. Septal cartilage with perichondrium and mucosa (type I collagen staining). Abundance of dark-brown staining type I collagen fibers can be seen in the perichondrium. No type I collagen present in the cartilage.

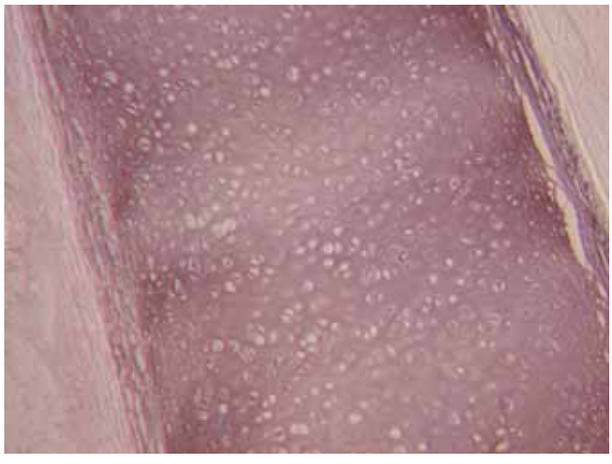

Figure 5. Septal cartilage with perichondrium (type II collagen staining). Collagen type II is observed in all zones of the cartilaginous matrix. Several somewhat darker staining areas reflecting a somewhat higher collagen II concentration are seen in the peripheral zones.

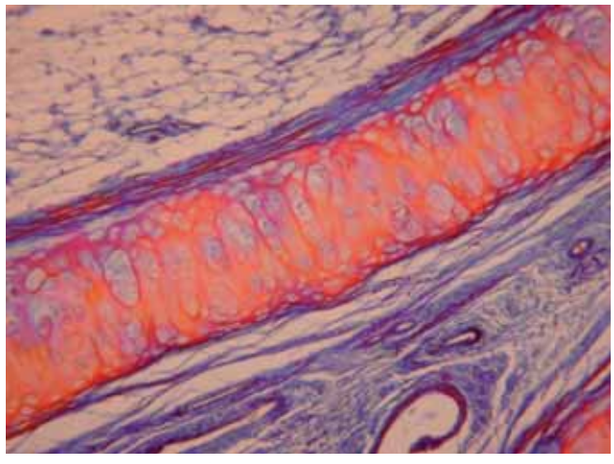

Figure 6. Lateral crus of lobular cartilage with perichondrium and adjacent connective tissue (Mallory-Cason staining). The morphology and orientation of the chondrocytes in the peripheral and central zones of the cartilage are similar to those in the septal cartilage. There is a distinct difference in the staining between the matrix of the septal cartilage and that of the lobular cartilage.

to mature collagen (Figure 3).
Immunohistochemistry staining for type I collagen reveals its complete absence in the cartilage but its high content in the perichondrium (Figure 4).
Type II is observed in all zones of the septal cartilage, with a somewhat higher concentration in certain peripheral areas (Figure 5).
Staining according to the methods of Verhoeff-van Gieson and Lawson does not reveal any elastic fibers in the septal cartilage but does demonstrate their presence in the septal perichondrium [8].

Lobular cartilage

Findings in the lateral crus of the lobular cartilage resemble those in septal cartilage in many respects. However, in the lobular cartilage the difference between the small flat chondrocytes lying parallel to the surface in the peripheral zones and the larger cells arranged palisade-like perpendicular to the cartilage surface in the central zones is more distinct (Figure 6).
Herovici staining demonstrates the presence of young ECM in the peripheral zones (staining bluish) (Figure 7).
Histochemistry on both type I and type II shows an absence of collagen I and a high content of collagen II in the cartilaginous matrix. These results are similar to the findings in septal cartilage is more distinct (Figures 8 and 9).

DISCUSSION

Most of the research on cartilage has been performed on articular cartilage. An excellent overview of the results was published by Buckwalter and Mankin [3]. They distinguished four different zones in articular cartilage. In the present study of the nasal cartilages, looking at cell morphology and arrangement

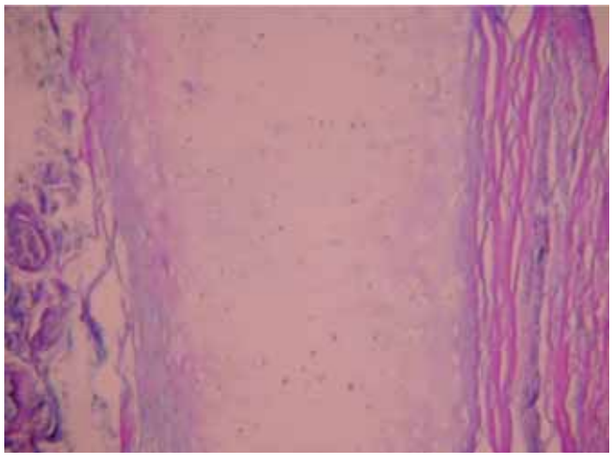

Figure 7. Lateral crus of the lobular cartilage with perichondrium (Herovici staining). The matrix of the peripheral zones stains bluish, indicating the presence of young collagen.

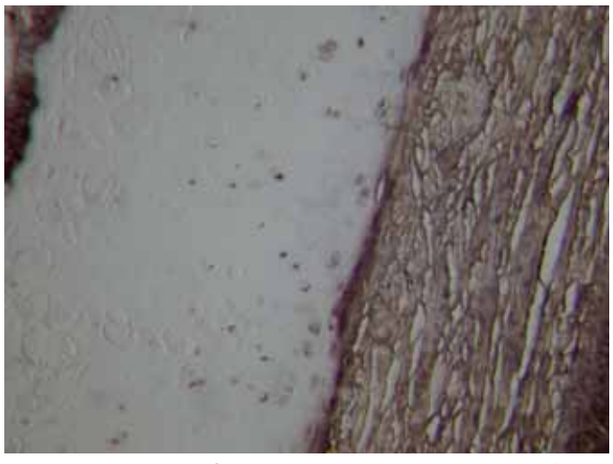

Figure 8. Lateral crus of lobular cartilage with perichondrium (type I collagen staining). High density of collagen I in the perichondrial fibers, no collagen type I in the matrix.

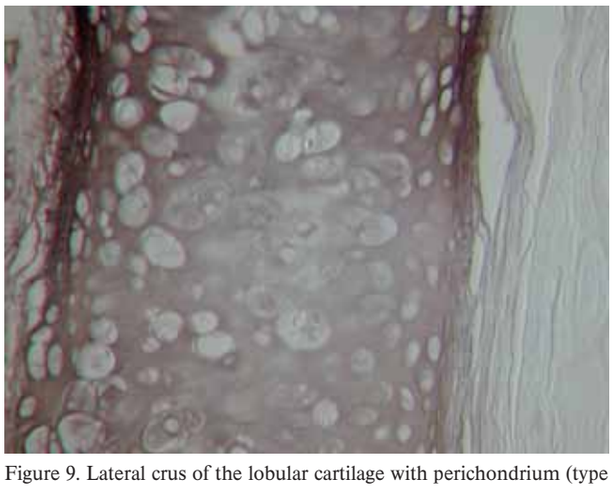

Figure 9. Lateral crus of the lobular cartilage with perichondrium (type II collagen staining). Collagen type II is observed in all zones of the cartilaginous matrix, particularly in the outermost zones.

as well as matrix composition, we found three zones that are consistent with the outer zones in articular cartilage. The fourth zone found in articular cartilage is related to the transition between cartilage and bone and is thus missing in nasal cartilage.

In both septal and lobular cartilage, we found a distinct transition from numerous small flat chondrocytes lying parallel to the cartilaginous surface to less numerous and larger ovaloid cells lying perpendicular to the surface. These differences represent the change in form and position between younger and mature cells. We cannot explain why the transition from small flat cells to larger ovaloid ones is accompanied by a 90-degree shift in cell orientation. Nor do we know why this association is more pronounced in the lateral crus of the lobular cartilage than in the septum. One could speculate that this shift in orientation is related to a special function of nasal cartilages, namely to provide a resistant yet pliable framework. Clearly, support is the primary function of septal cartilage. Lobular cartilage should have enough rigidity to keep the vestibule and the nasal valve area open, but at the same time it should allow mobility of the lateral nasal wall during respiration.

The extracellular matrix of both the septal and lobular cartilage was found to have a high density of type II collagen but no type I, as shown by our histochemical methods. This outcome is in agreement with observations made by others (3,4).

Like Üstünel et al.,(4) we found distinct differences between the peripheral and central zones, particularly in septal cartilage. Azan and Verhoeff-van Gieson staining demonstrated that the highest density of collagen is present in the peripheral zones. When applying Herovici and Lawson staining, the collagen in these outer areas appeared to contain more young collagen than the central zones, as was to be expected. Verhoeff-van Gieson and Lawson staining did not show elastic fibers in the cartilage.

In the matrix of the lobular cartilage, similar differences were found, although to a lesser degree. The high density of type II collagen in the septal cartilage, especially in the peripheral zones, supports the idea that one of the primary tasks of the septum is to provide stiffness to the external nose.

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