의학석사 학위논문

Morphologic and histologic changes of the septal cartilage after unilateral versus bilateral mucoperichondrial elevation

편측 및 양측 점막거상에 따르는 비중격 연골의 형태학적 및 조직학적 변화

2012 년 8 월

서울대학교 대학원

의학과 이비인후과학 전공

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Morphologic and histologic changes of the septal cartilage after unilateral versus bilateral mucoperichondrial elevation

By

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A thesis submitted in partial fulfillment of the requirements

for the degree of Master of Medicine (Otolaryngology)

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Approved by thesis committee

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Abstract

Introduction

The effect of unilateral versus bilateral elevation of mucoperichondrium on the morphological and histological changes of septal cartilage was evaluated.

Materials and methods

Eighteen mature New Zealand white rabbits were categorized into three groups according to the procedures they received: unilateral mucoperichondrial flap elevation (N = 6) vs bilateral mucoperichondrial flap elevation (N = 6) vs control (no flap elevation, N = 6). In each group, half of the subjects were sacrificed 3 months after the procedure and the other half 6 months after the procedure. Thickness was measured on the optical microscope image and histologic changes of chondrocyte and extracellular matrix were evaluated using H&E stain, Masson's trichrome stain, Alcian blue stain, and Verhoeff's elastic stain.

Results

The three groups showed similarity in terms of thickness of the cartilage. Histologic examination revealed that the chondrocyte numbers and dystrophic feature as well as the ratio of chondroblast

were not different among the 3 groups. Masson's trichrome staininng of extracellular matrix in bilateral group showed less dense collagen fibers than the unilateral group. Proteoglycan and elastic fiber composition did not differ among the 3 groups.

Conclusion

Bilateral mucoperichondrial flap elevation of the septum results in less dense collagen fibers in the extracellular matrix, although the thickness of cartilage, the characters of chondrocyte and chondroblast and other extracellular matrix component remain about the same.

Keywords: mucoperichondrial flap, septal cartilage, septoplasty

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List of abbreviation

H&E: hematoxylin & eosin

MT: Masson's trichrome

UMP: unilateral mucoperichondrial

BMP: bilateral mucoperichondrial

Group A: unilateral mucoperichondrial flap elevation group

Group B: bilateral mucoperichondrial flap elevation group

Introduction

Septoplasty is arguably the most common procedure performed by otorhinolaryngologists. As such, remarkable progress has been made over the past few decades relative to surgical technique.

In the 1880's, Krieg first introduced a novel procedure whereby deformed cartilaginous septum was segmentally resected *en bloc*, complete with overlying mucosa. Unfortunately, this early approach created potential for a sizeable perforation to develop long-term, marked by crusting, bleeding, and even worsened nasal obstruction. Improving upon Krieg's initial efforts, Freer later devised a mucosal flap elevation for removal of deviated septal cartilage, [2] but it was Boenninghaus who ultimately suggested that this concept be extended posteriorly to include the vomer and perpendicular plate of the ethmoid.[3, 4] Thereafter, incision and plane dissection have been pivotal in septoplasty.

More specifically, preservation of septal perichondrium is now recognized as critical to surgical success, by supplying the vasculature and mesenchymal cells needed to regenerate cartilage in surgical defects.[11, 12] Verwoed-Verhoeff, et al demonstrated that new cartilage is visible two weeks after submucosal

resection in young rabbits.[7] Freer and Ingal's window resection with mucosal flap elevation was likewise fueled by Krieg's *en-bloc* technique, conceived as yet another means of sparing mucosa and perichondrium for the purpose of repair.[2]

Aside from septal cartilage, the reparative capacity of perichondrium has been repeatedly documented in other areas of the head and neck. For example, cartilaginous grafts in treatment of laryngeal stenosis survive well when perichondrium is attached, while insufficient union and tissue reabsorption are long-term drawbacks when perichondrium is lacking.[13-15] It is therefore recommended that perichondrium be routinely included with free grafts of cartilage for optimal viability and intercartilaginous healing.[16]

Unilateral mucoperichondral (UMP) flap elevation is generally regarded as safe (for reasons above) and is accepted as a standard septoplasty procedure. On the other hand, bilateral flap elevation is sometimes warranted for adequate access and management of selective patients, especially those with severe deformity of the caudal end or dorsal septum.

Nonetheless, bilateral mucoperichondral (BMP) flap elevation has been discouraged until recently, owing to a perceived increase in the risk of injury

to perichondrium and a subsequently weakened septal cartilage that is prone to complications. It has been reported anecdotally (per observations at revision septoplasty) that BMP flap elevation results in thinner, more fragile cartilage, although an exhaustive search of the literature search has returned no formal data in this regard.

The intent of this study was to compare UMP and BMP flap elevations in terms of morphologic and histologic findings, using a rabbit animal model. In doing so, we hoped to provide a more scientific rationale for their preferential use.

Material and methods

All protocols and experimental design parameters were reviewed and approved by the Institutional Animal Care and Use Committee of the Seoul National University Hospital.

Eighteen mature New Zealand white rabbits (weight range, 3.17-4.70 kg) were randomly assigned to one of three groups as follows: UMP flap elevation (group A), BMP flap elevation (group B), and no flap elevation (controls).

Animal surgery

Prior to surgery, each animal received intramuscular injections, consisting of Zoltetil 10 mg/kg (tiletamine 125mg/cc, zolazepam 125mg/cc) and the Rumpun (2% xylazine) at a ratio of 1:2. The surgical field was infiltrated with combination of 2% lidocaine and 1:100,000 epinephrine. The columella was then incised horizontally (Fig. 1A), with angled scissors for sharp dissection of dorsal septum through an avascular plane (Fig. 1B). After incising caudal septum, a mucoperichondral flap was elevated posteriorly, as much as 4 cm

deep from the caudal end. Diligence was taken to preserve the cartilage and mucoperichondrium (Fig. 1C). For BMP flap elevation, a second incision was made on the contralateral septum, and the same dissection method was repeated (Fig. 1D). All bleeding was controlled with bosmin-soaked gauze.

The elevated flap was redraped and sutured through-and-through with 4-0. The columella was also closed with 4-0 vicryl. Intramuscular procaine penicillin (40,000 IU) was administered on a prophylactic basis for three consecutive days postoperatively.

Specimen harvest

In each group (n=6), half of the subjects (n=3) were sacrificed three months after surgery and the remainder six months after surgery. To secure septal cartilage, a midline dorsal incision was made from frontonasal suture to the nasal tip, exposing the entire nasal bone via lateral flap. Nasal bone was cut with Rongeur and removed. Septal cartilage was incised inferiorly along the nasal floor, and the cartilaginous septum was harvested *in toto*, after fracturing the nasal dorsum.

Preparation for histologic examination

Each specimen was trisected as shown (Fig 2) in order to compare anterior (5 mm), middle (20 mm) segments with posterior (40 mm) septum (all 5 mm in width).

Following standard fixation, tissues were dehydrated through a series of graded ethanol solutions and then embedded in paraffin. Sections made at 6 μm were subjected to routine hematoxylin and eosin (H&E) staining, Masson's trichrome (MT) stain for collagen, Alcian blue stain for proteoglycan content, and Verhoeff' stain for elastic fibers.

Thickness of cartilage

The thickness of septal cartilage was determined by optical microscope (Fig.3, left). Posterior septum was used to estimate preoperative thickness in calculating ratios of anterior and middle segments to posterior septum. The thickness of perichondrium was also measured on the each side of cartilage.

Histologic examination

At 100X magnification, mature chondrocytes (central zone) and chondroblasts (peripheral zone)[8, 9] were quantified in mid-longitudinal axis (dorsal to floor). A count of dystrophic chondrocytes was also obtained. Dystrophic change was defined as nuclear degeneration (karyolysis or karyopyknosis) and irregular cytoplasmic eosinophilia [10] (Fig. 4). Extracellular matrix was quantitatively graded by staining intensity using Image J® software (Fig. 3, right).

Statistical analysis

Statistical analysis was performed using the software package SPSS for Windows version 10.0 (SPSS Inc, Chicago, Ill). A nonparametric statistical test (Kruskal-Wallis) was used to assess multigroup differences.

Results

The rabbits were all male gender, with median weight of 4.18 kg. Animal weights were not significantly different among all groups (p=0.17).

Thickness of cartilage

The mean thickness of cartilage (by microscopy) is shown in Table 2. Values were similar for all groups, as were mean values (bilateral) of perichondrial thickness. Ratios of anterior and middle segments to posterior septum were not significantly different (p=0.46 and p=0.19, respectively).

Histologic examination

1. Chondrocytes and chondroblast

The number of chondrocytes and their proportion relative to chondroblasts did not differ significantly among groups (Table 3). Dystrophic chondrocytes (as quantified) were similar for all groups as well.

2. Extracellular matrix

With MT stain, collagen was clearly less abundant in group B (Fig. 6), corresponding with a significantly quantitatively higher value (lower intensity) (p=0.001, Fig. 5A). Otherwise, Alcian blue (p=0.08, Fig. 5B, 7) and Verhoeff's elastic stain (p=0.43, Fig 5C, 8), directed at proteoglycans and elastic fibers, failed to discriminate among groups.

Discussion

Septal cartilage is hyaline cartilage and is sensitive to trauma. Its cells and matrix are cloaked in and likely originate from perichondrium (mesenchymal cells, specifically). Mature, matrix-producing chondrocytes, constituting the sole cellular component of hyaline cartilage, are confined to the central zones, whereas chondroblasts align peripherally.[8]

Type II collagen and elastic fibers are found diffusely in the extracellular matrix of septal cartilage and are reinforced by proteoglycans, such as laminin and fibronectin.[17] Collagen fibers far outnumber elastic fibers, the latter seen chiefly in perichondrium.[9] In combination, however, these elements impart the stiffness or fragility displayed by distinct cartilaginous subtypes (hyaline, elastic, fibrocartilage) so variably suited for anatomic and functional requirements.[18] The mechanical properties of each subtype are governed by intercellular bridging and bonds formed between the matrix componentry

No difference in thickness of cartilage was observed among the three groups of our study. Given that preoperative thickness provides the best baseline reference value, a noninvasive means of precise preoperative measurement is

not practical. We instead utilized the posterior portion of septal cartilage, untainted by mucoperichondral flap, as the comparator. The ratio of anterior or middle segments of septum to the posterior portion was then indicative of relative postoperative change. The lack of discrepancy herein suggests that the flap elevation procedure does not readily affect septal cartilage.

In assessing overall cellularity of cartilage, we saw no differences among groups. Dystrophic change was used to gauge the degree of active cellular proliferation, with adequate manifestations signifying normal cell cycles and any increase attributable to ongoing regeneration and chondrocytic differentiation. Counts of chondrocytes/chondroblasts and the proportion of chondrocytes showing dystrophic change were similar for all three groups studied. On this basis, we concluded that mucoperichondral flap elevation has no visible effect on the viability of cartilaginous cells.

While elastic fibers of the extracellular matrix showed equivalency across groups, collagen fibers were decidedly less abundant with BPM vs UMP and controls. Collagen fibers are accentuated in fibrotic processes or scarring, contributing to tissue rigidity.[19] A loss of collagen fibers increases the fragility of cartilage and weakens its capacity for structural support.

There were no statistically significant differences among groups with Alcian blue stain, which is specific for proteoglycans. However the cartilage of bilateral mucoperichondrial flap elevation group showed lighter stain than the other groups, especially in central zone and showed lower intensity in quantitative analysis. (The mean value in Image J® software is inversely correlated with intensity of certain color) And there was tendency that the intensity is increased from bilateral flap elevation group to control. Even though we couldn't find any statistically significant difference, the loss of proteoglycan can be aggravated somewhat more by bilateral approach. If so, it also can contribute to the cartilage weakness by loss of mechanical support as above mentioned.

In summary, BMP flap elevation did not affect the thickness and cellularity of septal cartilage in our animal model. Similarly, extracelluar matrix remained intact, with the exception of diminished collagen fibers. While BMP flap elevation does not alter the viability of cells, it may affect their functionality, as indicated by specific changes in extracellular matrix.

Several publications have reported loss of volume and chondrocytic apoptosis with manipulation of septal cartilage, so that mucoperichondral elevation alone may adversely affect nasal septum.[21, 22] This implies that any

BMP elevation involving considerable manipulation of septal cartilage could be deleterious. Therefore, for the better quality of cartilage after septoplasty, it might be better to limit the bilateral approach.

Conclusions

In view of our findings, it is apparent that UMP flap elevation has little morphologic or histologic effect on underlying cartilage. While BMP elevation compares favorably with UMP overall, the decrease in collagenous matrix we observed may reduce the relative strength of septal cartilage.

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Table 1. Thickness of the septal cartilage.

		Thickness (mean, μm)				
		Anterior portion*	Mid-portion*	Posterior portion*	ant/post*	mid/post*
Group A	3 month	246.26	621.05	605.03	0.44	1.05
	6 month	255.75	559.43	620.68	0.41	0.90
Group B	3 month	224.27	650.53	655.67	0.35	1.00
	6 month	335.05	538.75	626.47	0.53	0.86
Control	3 month	284.48	658.04	543.69	0.61	1.11
	6 month	273.91	568.76	677.88	0.40	0.85

*$p > 0.05$, Kruskal-Wallis test (SPSS)

There were no signigicant difference among the 3 groups.

Table 2. The number of mature chondrocytes, chondrocytes with dystrophic change and chondroblasts

		Number				
		Chondrocyte*		Dystrophic change (%)*		Chondroblast (ratio†)*
			mean*		mean*	
Group A	3 month	87.3	88.3	7.0 (8.0)	7.8 (8.8)	55.4 (0.70)
	6 month	89.2		6.7 (7.5)		51.9 (0.65)
Group B	3 month	90.3	96.3	6.3 (7.0)	5.6 (5.8)	54.0 (0.66)
	6 month	102.2		4.8 (4.7)		51.0 (0.83)
Control	3 month	92.3	95.4	5.8 (6.3)	6.1 (6.4)	52.6 (0.73)
	6 month	98.5		6.3 (6.4)		51.1 (0.71)

*$p > 0.05$, Kruskal-Wallis test (SPSS)

†Percentage of chondroblast

There was no significant difference among the 3 groups.

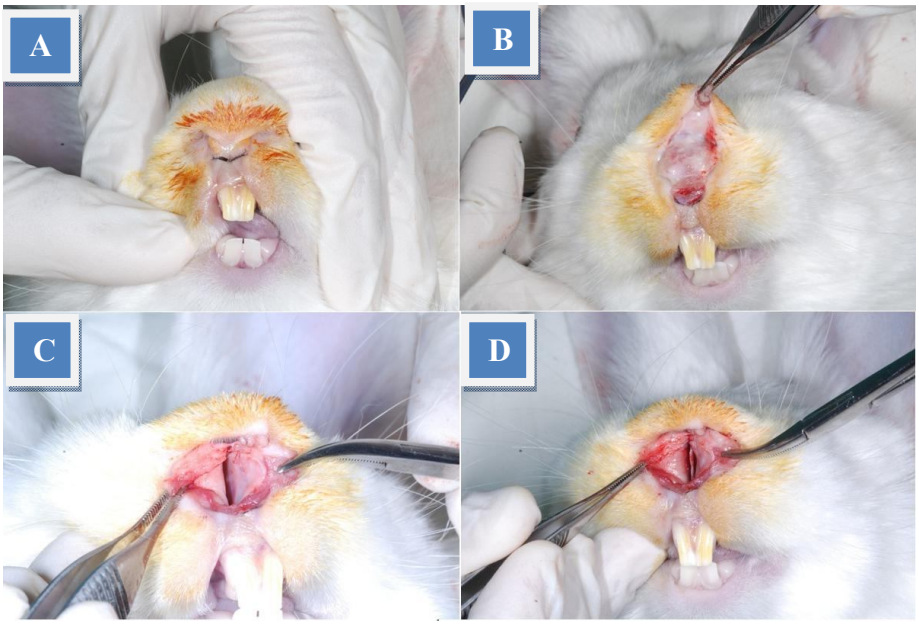


Figure 1. Operative procedures

A: columellar incision, B: elevation of the flap for exposure of the septum, C: elevation of unilateral mucoperichondrial flap, D: elevation of bilateral mucoperichondrial flap

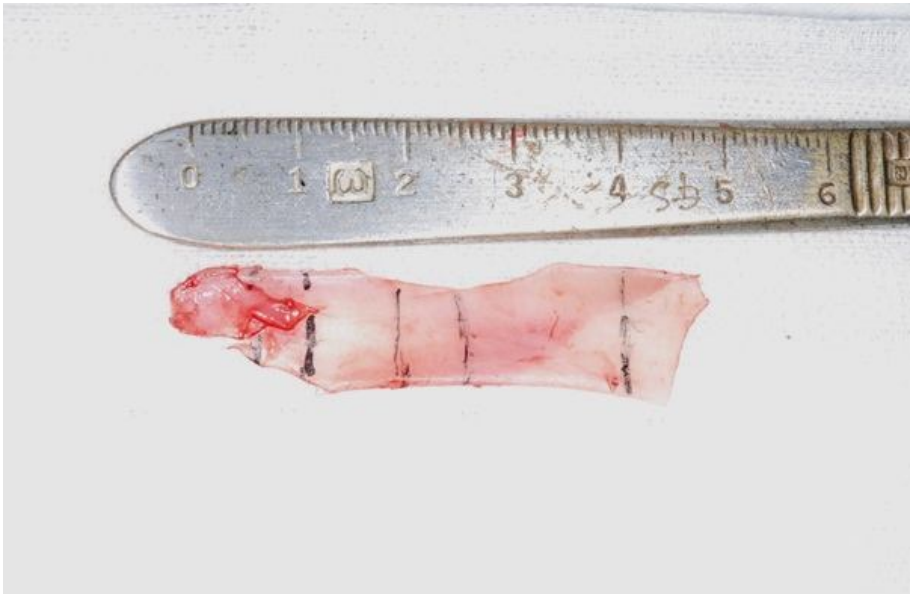


Figure 2. Preparation of the septal cartilage for histologic examination

Anterior portion (5 mm from the caudal end), mid-portion (20 mm from the caudal end), and posterior portion (40 mm from the caudal end) with a 5 mm width are marked.

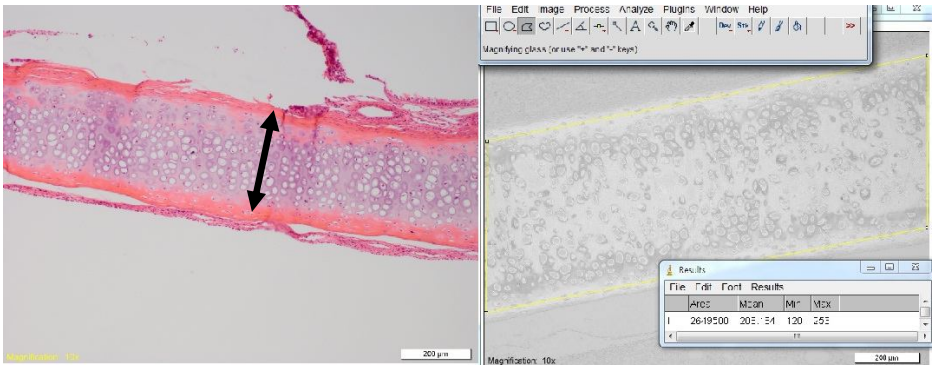


Figure 3. Measuring the thickness of cartilage and utilization of Image J® software for quantitative evaluation

left: optical microscopic image of cartilage (A4, anterior portion, H&E stain), Thickness was measured by drawing line running perpendicular to the long axis), right: blue color split image by Image J® software (A6, mid-portion, Alcian blue stain). The intensity of blue color is quantitatively measured with converting to 8-bit image after splitting the blue)

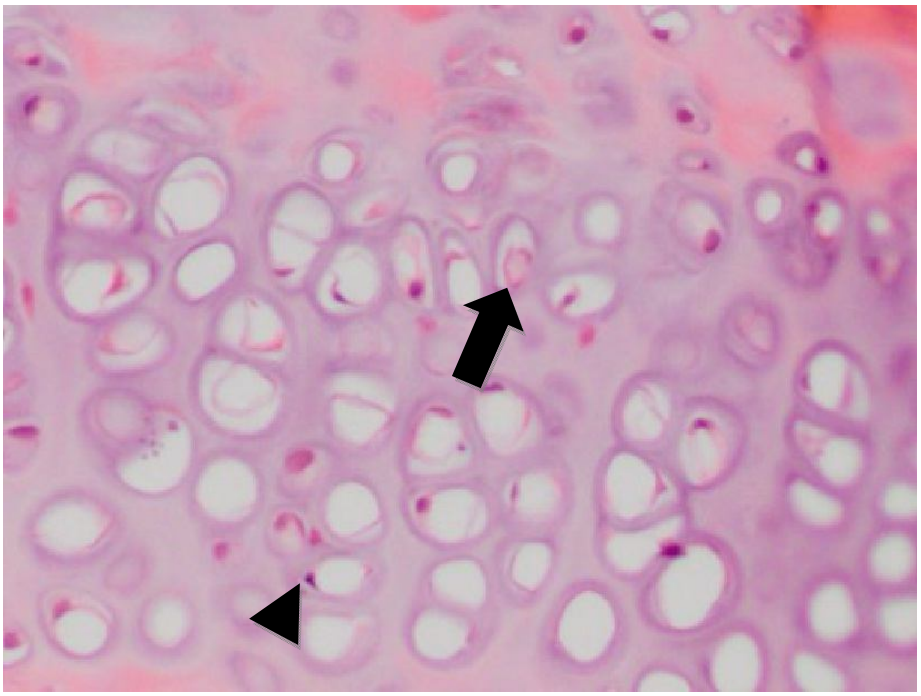
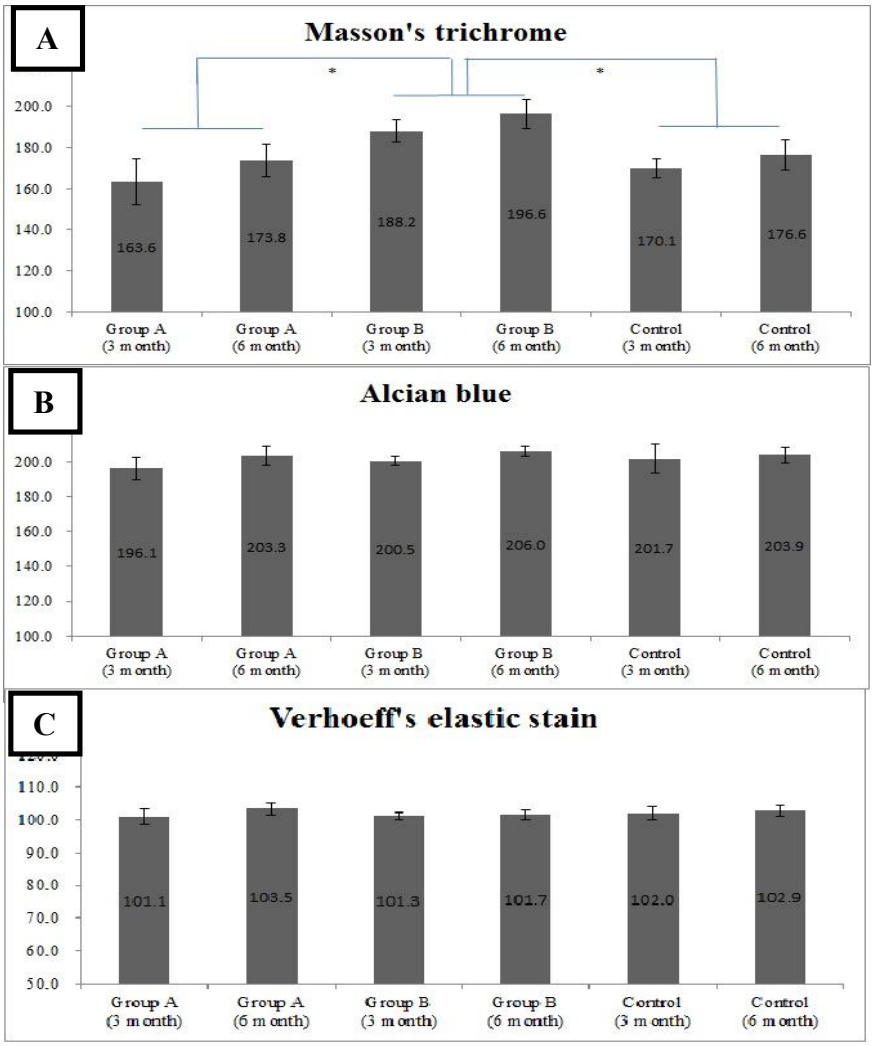


Figure 4. Dystrophic change of chondrocyte (A4, anterior portion)

arrow : karyolysis of chondrocyte, arrowhead : karyopyknosis of the chondrocyte



$*p < 0.001$

Figure 5. The mean value of intensity of extracellular matrix in Masson's trichrome stain (A), Alcian blue stain (B), Verhoeff's elastic stain (C) measured by Image J® software

The mean value and the intensity are inversely correlated. In Masson's trichrome stain, group B showed significant difference from group A and control ($p = 0.001$), however there was no significant difference among the 3 groups in Alcian blue stain and Verhoeff's elastic stain ($p = 0.08$, 0.43, respectively)

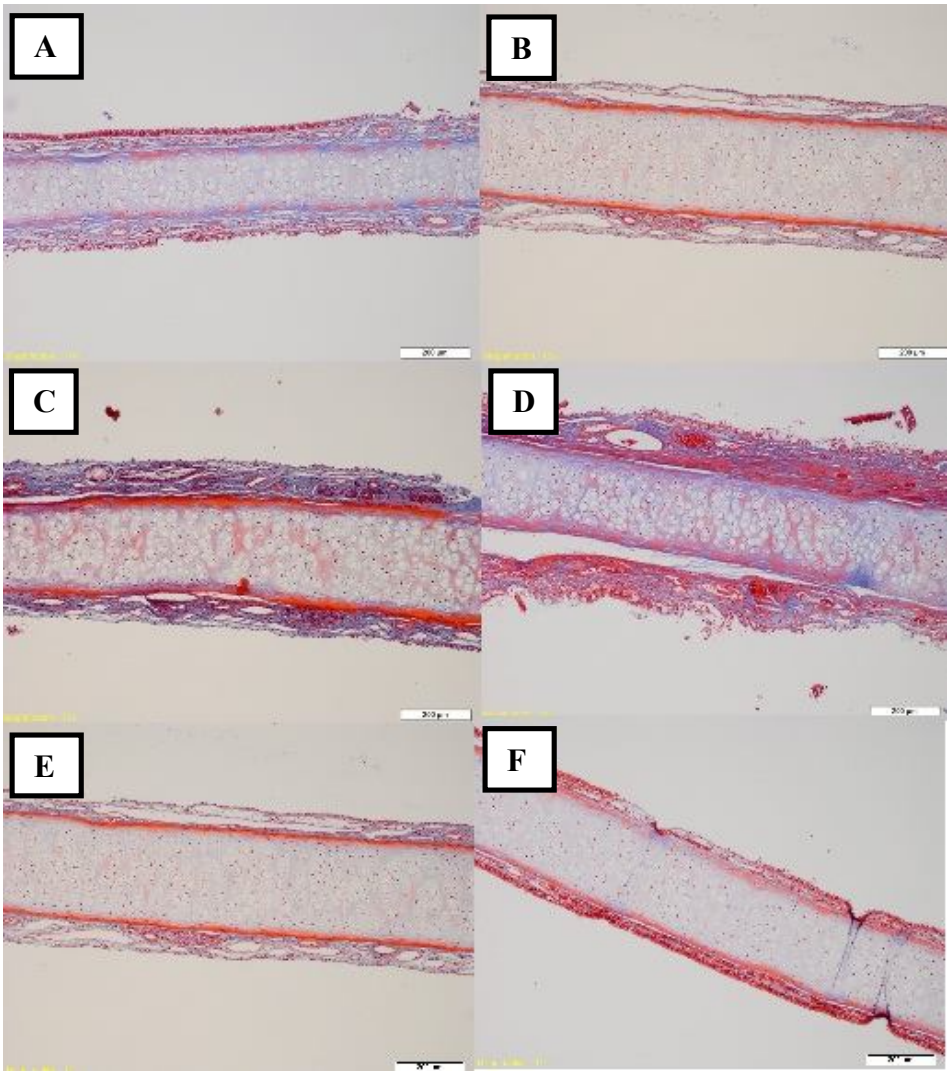


Figure 6. Extracellular matrix in septal cartilage (1)

(A: group A (3 month), B: group A (6 month), C: group B (3 month), D: group B (6 month), E: control (3 month), F: control (6 month), Masson's Trichrome stain, original magnification x100)

The Masson's trichrome is used to stain collagen fiber in septal cartilage which is shown in blue color. Bilateral mucoperichondrial flap elevation group (C, D) showed less amount of collagen tissue filled in the extracellular matrix than unilateral mucoperichondrial flap elevation group (A, B) and control (E, F)

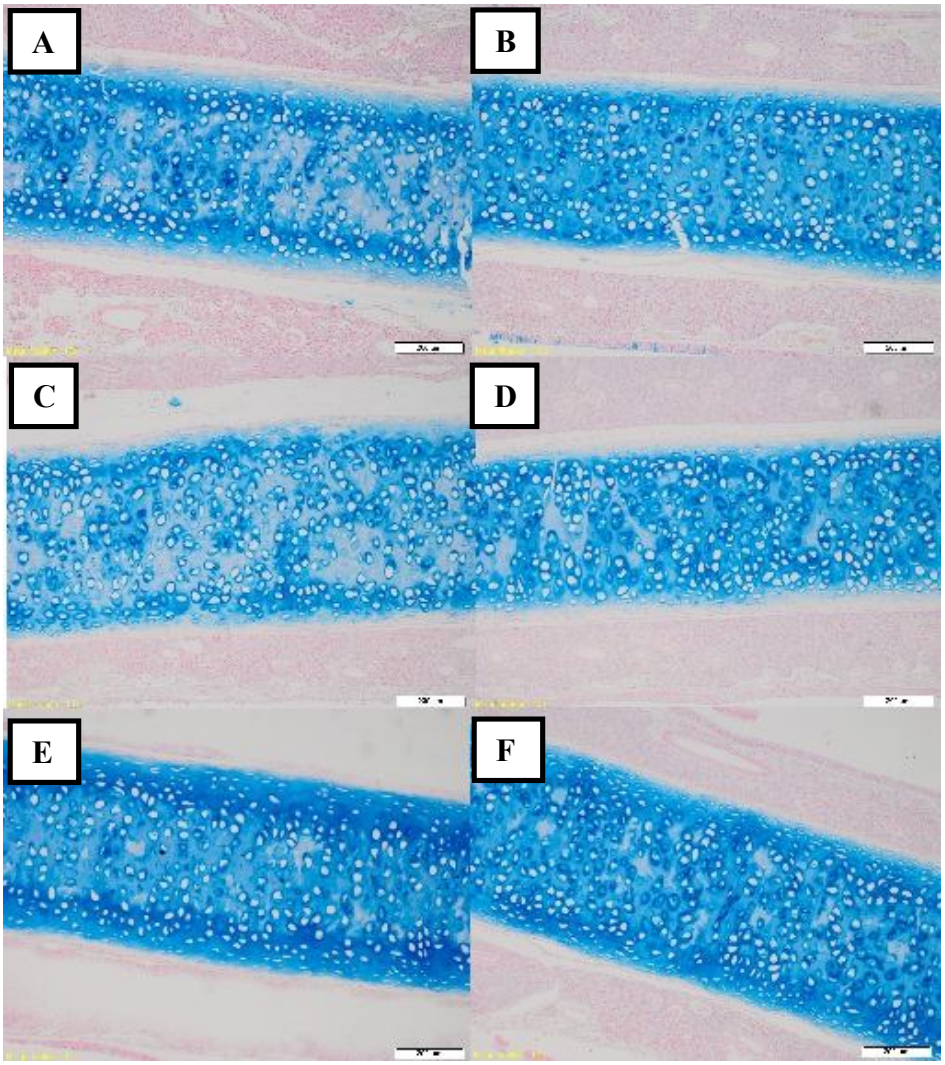


Figure 7. Extracellular matrix in septal cartilage (2)

(A: group A (3month), B: group A (6month), C: group B (3month), D: group B (6month), E: control (3month), F: control (6month), Alcian blue stain, original magnification x100)

Alcian blue stain is used for analysis of proteoglycan in septal cartilage which is shown in blue color. Bilateral mucoperichondrial flap elevation group, (C, D) especially 3 month group (C) looks paler than unilateral mucoperichondrial flap elevation group (A, B) and control (E, F)

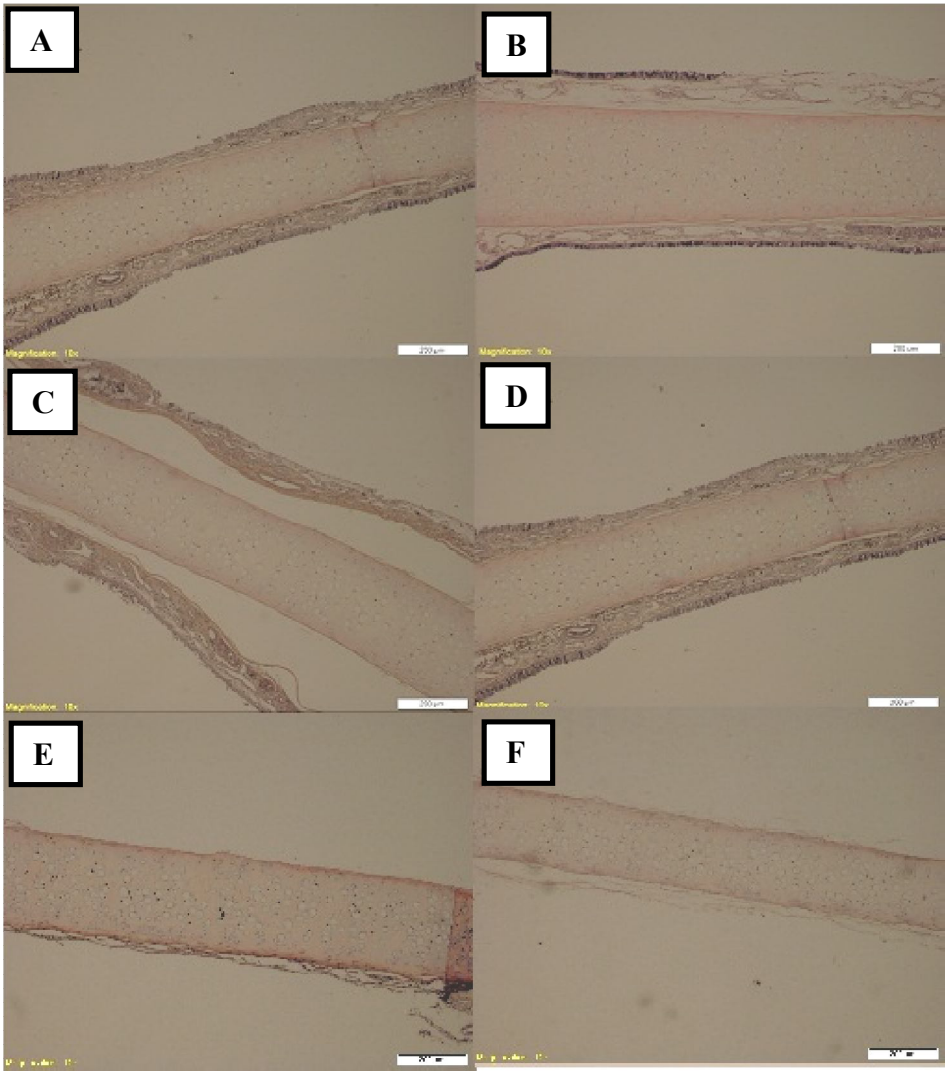


Figure 8. Extracellular matrix in septal cartilage (3)

(A: group A (3month), B: group A (6month), C: group B (3month), D: group B (6month), E: control (3month), F: control (6month), Verhoeff's elastic stain, original magnification x100)

The Verhoeff's stain is used for analysis elastic fiber in septal cartilage which is shown in brown color. All the groups show no significant difference

국문초록

서론

편측 및 양측 비중격 점막거상으로 인한 비중격 연골의 형태학적, 조직학적 변화를 연구하고자 하였다.

재료 및 방법

성숙한 수컷 가토 (New Zealand rabbit) 18마리를 술기에 따라 무작위로 편측 비중격 점막거상군, 양측 비중격 점막거상군, 아무 조작도 하지 않는 비교군의 세 그룹으로 나누었다. 각각의 그룹 중 3마리는 수술 3개월 뒤, 나머지 3마리는 6개월 뒤 희생시켰다. 비중격 연골의 형태를 관찰하고, 두께를 측정하였으며 H&E 염색, Masson’s trichrome 염색, Alcian blue 염색, Verhoeff’s elastic 염색을 통해 조직학적 소견을 비교 분석하였다.

결과

비중격 연골의 모양과 두께는 세 군간에 유의한 차이를 보이지 않았다. 비중격 연골 내 연골세포의 수, 세포이상변화 (dystrophic change), 연골세포에 대한 연골모세포의 비율도 세 군간에 모두

유의한 차이를 보이지 않았다. 반면 세포외 기질은 양측 점막거상을 시행한 군에서 연하게 염색되었고 특히 콜라젠 섬유(collagen fiber)가 유의하게 부족하였다.

결론

양측 비중격 점막거상군은 편측 점막거상군과 비교하여 비중격 연골의 형태, 두께 및 연골 내 세포의 특성에는 영향을 미치지 않지만 콜라겐섬유의 감소를 가져온다.

주요어: 비중격 점막거상, 비중격 연골, 비중격 교정술

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