Introduction

Septal deviation is one of the most common deformities of nose. To correct septal deviation, septoplasty is commonly performed porcedure.[1] Remarkable progress in septoplasty techniques have been achieved made during last decades. The deviation of cartilage portion can be corrected with submucosal resection of septal cartilage, however, it has some problems such as saddle nose, septal perforation, and difficulty in correcting severe deviation of caudal and dorsal portion. Therefore, “Cottle-tunnels” technique which could preserve cartilage was introduced by Cottle et al.[2] After that, Murakami et al developed a new cross-hatching technique which made full thickness incision at concave side.[3] Nowadays mucoperichondrial flap (MPF) elevation and cross-hatching incisions (CHI) are commonly used procedures for correcting deviated cartilaginous septum.[4] However, CHI may lead cartilage weakness, by full thickness cartilage incision itself, diminished perichondrial supporting or some other factors. We planned animal study to reveal the changes of cartilage after CHI.

Verwoed-Verhoeff, et al demonstrated that new cartilage is visible two weeks after submucosal resection in young rabbits.[5] And Lee et al reported bilateral mucoperichondrial flap elevation of the septum decreased collagen fibers and deposition of proteoglycan in the

extracellular matrix while maintaining the same chondrocyte cellularity, compared with unilateral flap elevation.[6] We have a question about how the cartilage would be healed after cross-hatching incision.

This study aimed to compare the effect of cross-hatching incision with unilateral or bilateral mucoperichondrial flap elevations on the morphologic and histologic findings of the septal cartilage using a rabbit model.

Materials and methods

All protocols and experimental design parameters were reviewed and approved by the Institutional Animal Care and Use Committee of the Boramae Medical Center(IACUC number 2012-0007).

Twenty-five mature New Zealand white rabbits (weight range, 3.17-4.70 kg) were randomly assigned to one of five groups as follows: unilateral mucoperichondrial flap (MPF) elevation without cross-hatching incisions group (unilateral MPF group, N = 5); unilateral MPF elevation with cross-hatching incisions group (unilateral MPFcCHI group, N = 5); bilateral MPF elevation without cross-hatching incisions group (bilateral MPF group, N = 5); bilateral MPF elevation with cross-hatching incisions group (bilateral MPFcCHI group , N = 5); no MPF elevation and no cross-hatching incisions group (control group, N = 5).

Animal surgery

The operation was performed as the same methods of the previous study.[6] Prior to surgery, each animal took intramuscular injections, consisting of Zoltetil 10 mg/kg (tiletamine 125 mg/cc, zolazepam 125 mg/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. Then, columella was incised horizontally, with angled scissors for sharp dissection of dorsal septum through an avascular plane. After incising caudal septum, a mucoperichondrial flap was elevated posteriorly, as much as 4 cm deep from the caudal end. Diligence was taken to preserve the cartilage and mucoperichondrium. For bilateral MPF elevation, a second incision was made on the contralateral septum, and the same dissection method was repeated. Cross-hatching incisions were performed with some vertical and horizontal line with a cottle knife. All bleeding was controlled with bosmin-soaked gauzes or bipolar cauterization. The elevated MPF was redraped and sutured through-and-through with 4-0 chromic gut. The columella was also closed with 4-0 vicryl. Intramuscular procaine penicillin (40,000 IU) was administered once postoperatively for prophylaxis of infection.

Specimen harvest

In each group, the subjects (n=5) were sacrificed three months after surgery. To secure septal cartilage, a midline dorsal incision was made from frontonasal suture to the nasal tip, exposing the entire nose. Nasal bone was cut with Rongeur and removed. Septal cartilage was incised inferiorly along the nasal floor, and the cartilaginous septum was totally harvested, after fracturing the nasal dorsum.

Preparation for histologic examination

Each specimen was trisected in order to compare anterior (5-10 mm), middle (20-25 mm) segments with posterior (40-45 mm) septum (all 5 mm in width). Follwing fixation with 4% buffered formaldehyde for 24 h, the 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's stain for elastic fibers.

Thickness of cartilage

The thickness of septal cartilage was determined by optical microscope(Olympus CX31RTSF, Tokyo, Japan). Posterior septum where perichondrium was not undermined, was used as a reference to investigate postoperative changes of thickness by calculating ratios of anterior and middle septa to posterior one.

Histologic examination

At x400 magnification, mature chondrocytes (central zone) and chondroblasts (peripheral zone)[5,7] were quantified in 10 randomly selected portion in each slide. A count of dystrophic chondrocytes was

also obtained with the same method. Dystrophic change was defined as nuclear degeneration (karyolysis or karyopyknosis) and irregular cytoplasmic eosinophilia.[8] The staining intensity of extracellular matrix was quantitatively graded by an investigator blinded to study groups, using Image J® software. The staining intensity is quantitatively measured with converting to 8-bit image after splitting the interested color. Because the more strongly stained color shows brighter after converting the image, the mean value of intensity and the amount of extracellular matrix are inversely correlated.

Statistical analysis

Statistical analysis was performed using the software package SPSS for Windows version 19.0 (SPSS Inc, Chicago, IL, USA). A nonparametric statistical test (Kruskal-Wallis) was used to assess multigroup differences. The Mann-Whitney U test was applied to determine statistical differences between two groups. Statistical significance was assumed at $P < .05$ for all variables. Data was expressed as the mean±standard deviation (SD).

Results

Among twenty-five rabbits, three were died during or immediately after surgery. The rabbits were all male gender, with median weight of 3.44 kg. Animal weights were not significantly different among all groups (p=0.62).

Gross morphology and thickness of cartilage

There was no prominently curved or warped septum among all specimens. The cross-hatching incision scar site showed no difference compared with control area on naked eye inspection. The mean values of the thickness of cartilage measured under microscope showed no significant difference among all five groups (Table 1). Ratios of anterior and middle segments to posterior septum were not significantly different (p=0.94 and p=0.40, respectively).

Histologic examination

The numbers of chondrocytes were similar among five groups (Table 2). Proportion of dystrophic chondrocytes showed no significant difference in all five groups.

With Masson's trichrome stain, collagen was not significantly different among all five groups, according to the intensity measured by

Image J software (p=0.304, Fig. 2A, Fig. 3). The Alcian blue stain was more dense in no cross-hatching groups and control group than the cross-hatching incision groups and bilateral MPF group, with significant difference (p=0.005, Fig. 2B, Fig. 4). Verhoeff's elastic stain (p=0.417, Fig 2C, Fig 5), which is directed at elastic fibers, failed to show any significant differences among groups.

Discussion

On previous study, bilateral mucoperichondrial flap elevation did not affect the thickness, cellularity of septal cartilage. For extracellular matrix, collagen fiber and proteoglycan content were decreased, but elastic fiber was not decreased.[6] This study is a follow-up study of previous study. The difference on study process was the sacrifice schedule. Previously, the sacrifice was carried on both three and six month after surgery, but this time it was performed only three month after surgery. All the other procedures were identically processed.

On inspection of gross morphology of obtained specimen, there was no prominent curving or warping on septal cartilage. The cross-hatching incision site seemed to be similar with other site. At a time of sacrifice, some adhesion was detected on mucoperichondrial incision site. However, there was no remarkable finding on histologic exam.

In assessing the thickness of cartilage, we found no differences among five groups. Overall cellularity of cartilage showed no difference. And elastic fibers in the extracellular matrix also showed no significant difference among all groups. These results were compatible findings compared with last study.

The proteoglycans, which were detected with Alcian blue stain, were distinctly less abundant in the flap elevation or cross-hatching group compared with control group. Unilateral flap elevation made more decreased in proteoglycan than control group, bilateral or cross-hatching brought more decreased in proteoglycan than only unilateral flap elevation group. However, there was similarity in the amount of proteoglycan among bilateral flap elevation group, unilateral flap elevation with cross-hatching group and bilateral flap elevation with cross-hatching group. That implies bilateral flap elevation may affect the damage to cartilage as much as cross-hatching incision. Moreover, bilateral flap elevation itself brings already enough damage to proteoglycan, simultaneous cross-hatching incisions don't affect additional decrease of the proteoglycan level. Proteoglycans are glycosylated proteins which have covalently attached highly anionic glycosaminoglycans. Many forms of proteoglycans are present in all extracellular matrices of connective tissues. The predominant proteoglycan present in cartilage is the large chondroitin sulfate proteoglycan 'aggrecan'. Aggrecan serves a primary role providing the osmotic resistance necessary for cartilage to resist compressive loads. Other species of proteoglycans like decorin, perlecan, have been identified and they demonstrated diverse biological functions.[9-11]

In this study, collagen fibers, which were stained with Masson's trichrome, showed no significant difference regardless of flap elevation or cross-hatching. That is the result that does not correspond with last study. One of the differences from last study is sacrifice time, three months after surgery, whereas six months in the last study. In culture study, it was proven that inflammatory cytokine interleukin-1 (IL-1) induces both proteoglycan decrease and type II collagen denaturation in porcine, bovine, and human cartilages.[12-14] And there is some evidence that a loss of proteoglycan in cartilage precedes to type II collagen.[15,16] IL-1α initially induced a decrease in tissue proteoglycan content in nasal cartilage. The proteoglycan loss was followed by IL-1α–induced cleavage of type II collagen by collagenase, which was often reflected by increased denaturation.[17] Therefore, short surgery to sacrifice period may lead no difference of collagen fibers among flap elevation or cross-hatching incision groups. Future study with immunohistochemistry may help to define mechanism of our result better.

In summary, cross-hatching incision did not affect the cellularity and thickness of septal cartilage in our in a rabbit model. Amount of elastic fiber and collagen fiber maintained three months after cross-hatching

incision. However, proteoglycan content decreased by cross-hatching incision or elevating MPF. In process of time, collagen fibers would decrease after proteoglycan degradation. Although cross-hatching incision does not modify the viability of cells, it may affect their biochemical future, as showed by specific changes in extracellular matrix.

Conclusions

In our animal study, cross-hatching incisions of the septum decreased the deposition of proteoglycan in the extracellular matrix while maintaining the same chondrocyte cellularity and extracellular matrix. It may reduce biochemical property and structural support of septal cartilage.

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References

1. Min Y, Jung H, Kim C. Prevalence study of nasal septal deformities in Korea: results of a nation-wide survey. Rhinology 1995; 33:61.
2. Cottle MH, Loring RM, Fischer GG, Gaynon IE. The maxilla-premaxilla approach to extensive nasal septum surgery. AMA archives of otolaryngology 1958; 68:301-313.
3. Murakami WT, Wong LW, Davidson TM. Applications of the biomechanical behavior of cartilage to nasal septoplastic surgery. The Laryngoscope 1982; 92:300-309.
4. Bailey BJ. Nasal septal surgery 1896-1899: transition and controversy. Laryngoscope 1997; 107:10-16.
5. Verwoerd-Verhoef HL, ten Koppel PG, van Osch GJ, Meeuwis CA, Verwoerd CD. Wound healing of cartilage structures in the head and neck region. International journal of pediatric otorhinolaryngology 1998; 43:241-251.
6. Lee DY, Kim DW, Mangoba DC, Kim DY, Jin HR. Morphological and histological changes of the septal cartilage after unilateral versus bilateral mucoperichondrial flap elevation. American journal of rhinology & allergy 2013; 27:62-66.
7. Bleys R, Popko M, De Groot J, Huizing EH. Histological structure of the nasal cartilages and their perichondrial envelope II. The perichondrial envelope of the septal and lobular cartilage. Rhinology 2007; 45:153.
8. Popko M, Bleys R, De Groot J, Huizing EH. Histological structure of the nasal cartilages and their perichondrial envelope I. The septal and lobular cartilage. Rhinology 2007; 45:148.
9. Yanagishita M. Function of proteoglycans in the extracellular matrix. Acta pathologica japonica 1993; 43:283-293.
10. Perrimon N, Bernfield M. Cellular functions of proteoglycans—an overview *Seminars in cell & developmental biology*: Academic Press, 2001:65-67.
11. Knudson CB, Knudson W. Cartilage proteoglycans. Seminars in cell & developmental biology 2001; 12:69-78.
12. Henderson B, Edwards J, Pettipher E. Mechanisms and models in rheumatoid arthritis. Academic Press, 1995.
13. Dodge GR, Poole AR. Immunohistochemical detection and immunochemical analysis of type II collagen degradation in human normal, rheumatoid, and osteoarthritic articular cartilages and in explants of bovine articular cartilage cultured with interleukin 1. Journal of Clinical Investigation 1989; 83:647.
14. Mort JS, Dodge GR, Roughley PJet al. Direct evidence for active metalloproteinases mediating matrix degradation in interleukin 1-stimulated human articular cartilage. Matrix 1993; 13:95-102.

15. Kozaci LD, Buttle DJ, Hollander AP. Degradation of type II collagen, but not proteoglycan, correlates with matrix metalloproteinase activity in cartilage explant cultures. Arthritis & Rheumatism 1997; 40:164-174.
16. Cawston T, Ellis A, Humm G, Lean E, Ward D, Curry V. Interleukin-1 and oncostatin M in combination promote the release of collagen fragments from bovine nasal cartilage in culture. Biochemical and biophysical research communications 1995; 215:377-385.
17. Billinghurst RC, Wu W, Ionescu Met al. Comparison of the degradation of type II collagen and proteoglycan in nasal and articular cartilages induced by interleukin-1 and the selective inhibition of type II collagen cleavage by collagenase. Arthritis and rheumatism 2000; 43:664-672.

Table1. Thickness of the septal cartilage.

	Thickness (mean, ㎛)				
	Anterior portion*	Mid-portion*	Posterior portion*	ant/post*	mid/post*
unilateral flap elevation	244.6	602	586.4	0.42	1.03
unilateral flap elevation + Cross-hatching	263	590.5	605.5	0.43	0.98
Bilateral flap elevation	251.8	600	588.8	0.43	1.02
Bilateral flap elevation + Cross-hatching	253.3	600.3	617.5	0.41	0.97
Control	250.8	584	593.4	0.42	0.98

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

There was no significant difference among the 5 groups.

Table2. The numbers of mature chondrocytes, chondrocytes with dystrophic change and chondroblasts.

	Number (mean)		
	Chondrocyte*	Dystrophic change (%)*	Chondroblast (%)*
unilateral flap elevation	266.4	19.8(7.4)	191.4(71.8)
unilateral flap elevation + Cross-hatching	270	22(8.1)	204.8(75.6)
Bilateral flap elevation	264.8	19.5(7.4)	196(74.5)
Bilateral flap elevation + Cross-hatching	260	20.8(7.5)	199(77.6)
Control	273.2	20.5(7.6)	181.4(66.9)

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

There was no significant difference among the 5 groups.

Figure legends

Figure 1. Operative procedures and preparation of the septal cartilage for histologic examination

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

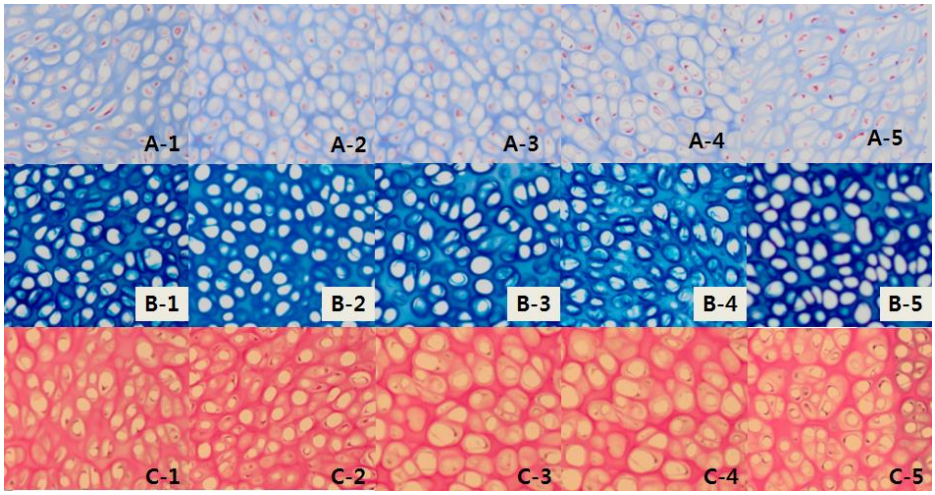

Figure 2. The comparison of the component of the extracellular matrix in each group(1st, unilateral group; 2nd, unilateral with cross-hatching group; 3rd, bilateral group; 4th, bilateral with cross-hatching group; 5th, control group).

(A) Masson's trichrome stain (original magnification, x400) shows collagen fiber in blue color. All of the groups show no significant difference.

(B) Alcian blue stain (original magnification, x400) shows proteoglycan in blue color. Manipulated groups shows less amount of proteoglycan than control group.

(C) Verhoeff's stain (original magnification, x400) shows elastic fibers in brown color. All of the groups show no significant difference.

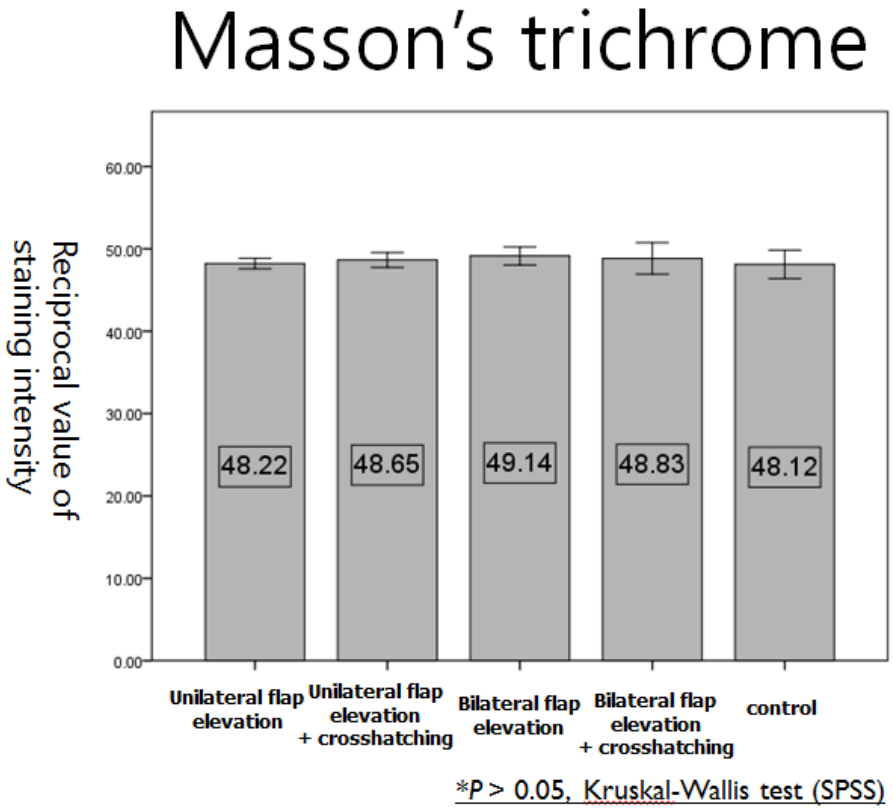

Figure 3. The mean value of intensity of the extracellular matrix in Masson's trichrome stain.

There was no significant difference among the 5 groups in Verhoeff's elastic stain(p = 0.304).

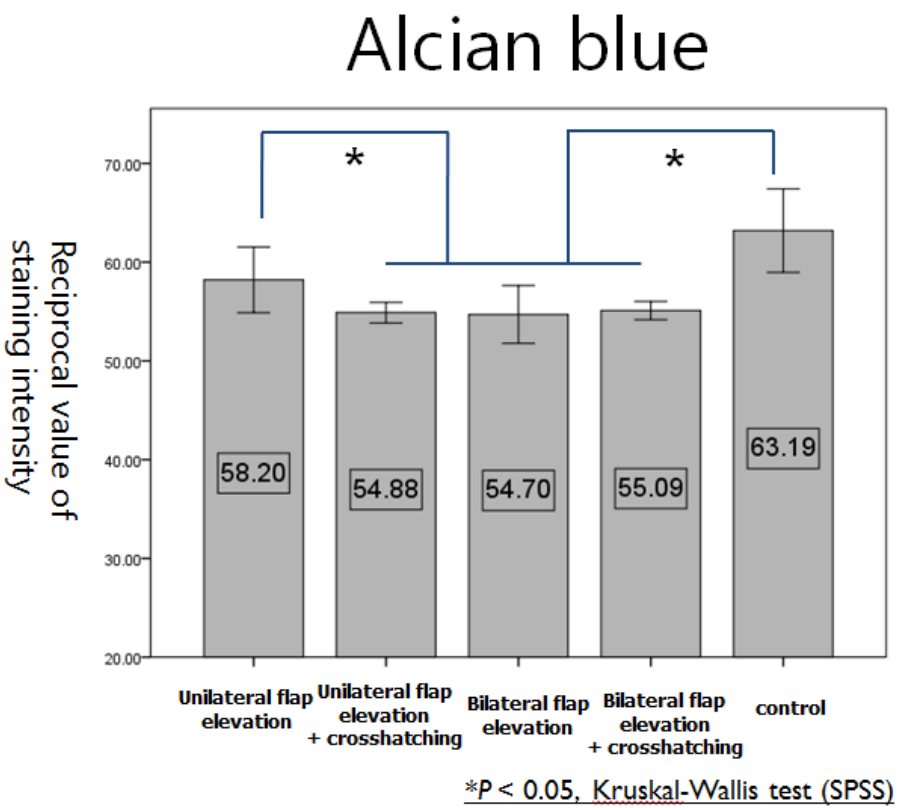

Figure 4. The mean value of intensity of the extracellular matrix in Alcian blue stain (*statistically significant)

In Alcian blue stain, 5 groups showed significant difference(p = 0.005, Kruskal-Wallis test).

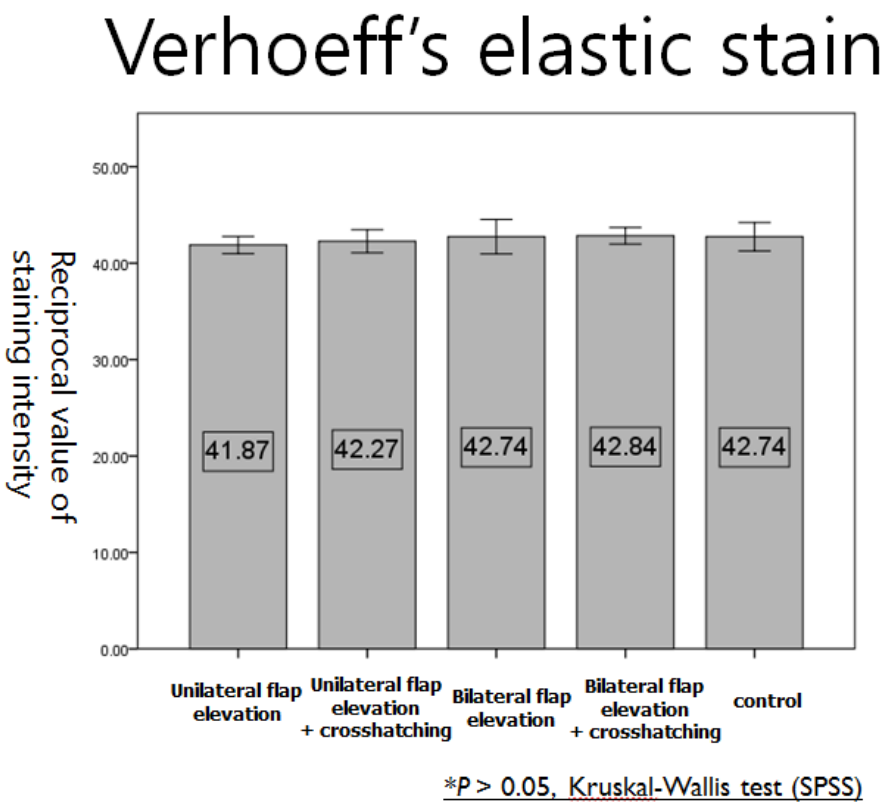

Figure 5. The mean value of intensity of the extracellular matrix in Verhoeff's elastic stain

There was no significant difference among the 5 groups in Verhoeff's elastic stain(p = 0.417).

국문초록

서론: 비중격 연골의 교차절개 후 연골의 형태학적, 조직학적 변화를 연구하고자 하였다.

재료 및 방법: 성숙한 수컷 가토 (New Zealand rabbit) 25마리를 술기에 따라 무작위로 편측 비중격 점막거상군, 편측 비중격 점막거상 및 교차절개를 시행한 군, 양측 비중격 점막거상군, 양측 비중격 점막거상 및 교차절개를 시행한 군, 아무 조작도 하지 않는 비교군의 5 그룹으로 나누었다. 수술 3개월 뒤 가토를 희생시켜 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 다섯 군간에 유의한 차이를 보이지 않았다. 비중격 연골 내 세포이상변화 (dystrophic change), 연골세포에 대한 연골모세포의 비율, 콜라겐 섬유(collagen fiber), 탄력 섬유 (elastic fiber) 도 다섯 군간에 모두 유의한 차이를 보이지 않았다. 반면 세포외 기질 중 프로테오글리칸(proteoglycan)은 점막거상 혹은 교차 절개 시

유의하게 연하게 염색되었다.

결론: 교차 절개시 비중격 연골의 형태, 두께 및 연골 내 세포의 특성에는 영향을 미치지 않지만 프로테오글리칸의 감소를 가져온다.

주요어: 비중격 점막거상, 비중격 연골, 비중격 교정술, 교차절개

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