석 사 학 위 논 문

Functional and Histologic Analysis of Popular Techniques for Muscle Suture in Cleft Lip and Palate

계 명 대 학 교 대 학 원

의 학 과

김 재 훈

지도교수 정 운 혁

2 0 2 1 년 2 월

Functional and Histologic Analysis of Popular Techniques for Muscle in Cleft Lip and Palate

지도교수 정 운 혁

이 논문을 석사학위 논문으로 제출함

2021년 2월

계명대학교 대학원
의 학 과

김 재 훈

김재훈의 석사학위 논문을 인준함

주 심 최 재 훈

부 심 정 운 혁

부 심 조 태 희

계 명 대 학 교 대 학 원

２０２１년 ２월

Table of Contents

List of Figures

1. Introduction

Cleft lip and palate ranges from complete to concealed type, such as microform for cleft lip and submucosal type for cleft palate. Orbicularis oris muscle (OOM) and levator veli palatine (LVP) muscles fail to join at cleft site in every cleft lip/palate whatever concealed type. This is histologic stigma for cleft lip and palate, even in microform cleft lip and submucosal cleft palate. One of important aim for cleft lip/palate repair is restoration of diastasis muscle on all of various surgical technique.[1-3] Philtrum is unique struc ture that composed with ridge called as philtral column and central depression called as philtral dimple. To mimic the shape of philtral column and dimple in cleft lip, cleft surgeon utilized various muscle suture technique from interrupted suture[4] to buried horizontal mattress suture,[5] overlapping,[6] coronal splitting,[7] interdigitating,[8] and splitting with folding technique.[9] Likewise, overlapping technique of levator veli palatini muscle have gained popularity to strengthen velopharyngeal closure force in palatoplasty.[10] Indeed, a huge effort has been made to improve surgical outcome by manipulating muscle in cleft lip/palate surgery.

Although it has been reported several suture technique of muscle in cleft lip/palate repair, majority of studies analyzed surgical outcomes by subjective method or anthropometric analysis instead of an analysis of muscle healing itself.[6-9,11,12] Human study has some limitation to evaluate muscle regeneration grossly or histologically after surgery. Nevertheless, experimental evaluation using animal was also rarely reported to date. Therefore, we performed animal study to evaluate three typical muscle suture technique applied in cleft lip/palate surgery; simple interrupted,

overlapping, and splitting-interdigitating suture. Our study evaluated functional outcomes of muscle action and histological outcomes of muscle regeneration in animal study.

2. Materials and Methods

2.1. Animal model

A total thirty adult male Sprague-Dawley rats (ORIENT BIO Inc, Seongnam, Republic of Korea) weighing 230 to 260g were used in this study. All experimental procedures conducted in the study were approved by The University Animal Care Committee for Animal Research of Keimyung University. General anesthesia was conducted via intraperitoneal injection a zolazepam tiletamine mixture (30 mg/kg, Zoletil®; Virbac, Carros, France) and xylazine (10 mg/kg, Rompun®; Bayer, Leverkusen, Germany). A small linear incision was made on right axilla skin. The triceps muscle was exposed via blunt dissection and cut horizontally in the midline using no.15 blade. Then the muscle was repaired with three different suture technique; simple interrupted suture (Simple group, n=10), overlapping suture (Overlapping group, n=10), and splitting-interdigitating suture (Splitting group, n=10; Fig. 1).

2.2. Grip strength test

Forelimb grip strength was measured bilaterally using the grasping test described by Bertelli and Mira.[13] The test is carried out by holding the rat's tail while they grip an aluminium bar, and pulling it back until the rat misses the grip. The customized aluminium grip bar was connected to a digital display (TRIPOD digital push-pull gage SF-50,

AERMANDA, China). The test was repeated twice and the average grip force per weight (N/100g) was included in the statistical analysis. The person carrying out the testing was blind to measurements (Supplementary digital content 1). Grip strength test were recorded repetitively in 5 SD rats except which is killed for biopsy at Week 1. Grip strength test was performed before the surgery for baseline test and post-operative Week 1, 2, 4, and 8.

2.3. Spatial gait symmetry test

Gait test was performed to analyze the range of motion as that strides were compared between operated side and non-operated side anterior feet. First, right foot was painted with a thin layer of water soluble pink dye and blue on left foot. Rats were placed at the open end of gait passage which is 60 cm in length covered with a sheet of clear plastic and allowed to proceed to the opposite ends without an external stimulus. As rats voluntarily proceed the gait passage, each steps were recorded on underlying paper sheet. Spatial symmetry was calculated as the ratio of step length left foot to right foot and stride length left foot to left foot (Supplementary digital content 2). For a spatially symmetric gait, spatial symmetry will be approximately 0.5, indicating that the right foot-strike is spatially centered between two left foot-strikes. Variance from spatial symmetry means that they may have problems of stretching right forelimb or shifting their weight from right forelimb to the other. Spatial gait symmetry test were recorded repetitively in 5 SD rats except which is killed for biopsy at Week 1. Spatial gait symmetry test were recorded at post-operative Week 1, 2, 4, and 8.

2.4. Histologic analysis

After SD rats were killed using carbon oxide chamber, the triceps muscle was isolated from 5 rats for each group, on post-operative week 1 and 8 respectively for histologic and immuno-histochemistric examination.

Specimens were formalin fixed, paraffin embedded, and sectioned into 5μm thick slices. Standard hematoxylin and eosin staining was performed to determine inflammation cellular response and general morphologic features. Tissue samples were observed using a light microscope. All slides were reviewed by a pathologists blinded from the study.

2.5. Western blot analysis

Rabbit polyclonal antibodies against IL-1 (1:1000; Santa Cruz, CA, USA), TNF-α (1:1000; Abcam) were used as primary antibodies; glyceraldehyde-3-phosphate dehydrogenase (GADPH), a house keeping genes were also determined with specific antibodies (1:4000). Detection of immuno-reactive bands was performed by image scan using a Fusion FX(VILBER, France)

2.6. Immunohistochemistry

Formalin-fixed, paraffin-embedded, 5μm thick tissue sections underwent automated immunohistochemistry by Ventana BenchMark XT immunostainer (Ventana Medical Systems, Tucson, AZ) using Mouse Anti-TNF alpha antibody(abcam, ab1793) and Mouse Anti-IL-1beta antibody(santacruz, sc52012) with 32-min incubation time, diluted to 1:10000 for TNF alpha antibody and 1:5000 for IL-1beta antibody. Then visualized by Optiview DAB IHC Detection Kit(Ventana, 760-700) with amplification. The specimens were counterstained with Ventana kit (Hematoxylin 4′ + Bluing Reagent 4′). Antibody immunopositivity was evaluated on a scale from 0 to 3 (0, scanty; 1, some; 2, many; 3, extensive) by a pathologist blinded to the study.

To detect regeneration of muscle fiber, fluorescence immunohistochemistry was performed with anti-Laminin (1:100, Novusbio) and anti-MyoD antibodies(1:100, Santacruz). Each specimens were also stained with DAPI to visualize the nucleus in mitotic cells. The immunohistochemistry was carried out using typical methods.

2.7. Statistical analysis

Spatial gait symmetry and grip strength were statistically analyzed using two-way ANOVA. Staining intensity in immuonohistochemistry was analyzed by nonparametric analysis using the Kruskal-Wallis test followed by Dunn's multiple comparisons test in Graphpad Prism 8^{TM} (GraphPad Software Inc, California, USA). Differences with a value of $p < 0.05$ was considered statistically significant.

3. Results

3.1. Simple suture and overlapping suture demonstrates stronger muscle power but similar range motion compared with splitting group

Reduced grip force (N/100g) was identified by postoperative week 2 regardless of suture technique. Given that grip force per weight gradually increases as rats grow in size, they seemed to regain grip strength in relation to normal rats at week 4. But there is no significant difference between three groups until Week 4. At week 8, grip force per weight of simple group (median, 3.49; IQR, 3.28 to 3.66) and overlapping group (median, 3.3; IQR, 3.17 to 3.47) was significantly strong compared with splitting group (median, 2.91; IQR, 2.76 to 3.05) in two-way ANOVA test (simple vs. splitting, $p^{**} = 0.009$; overlapping vs. splitting, $p^{*} = 0.011$; Fig. 2). This indicate that muscle which is treated with simple and overlapping suture techniques induce better physiologic recovery than splitting group.

Spatial symmetry is used to evaluate geometric pattern of foot strike. Whereas gaits were almost symmetric with median value of 0.5 (IQR, 0.47 to 0.51) in simple suture, overlapping and splitting suture technique were spatially asymmetric with value of 0.55 (IQR, 0.53 to 0.62) and 0.51 (IQR, 0.45 to 0.63) respectively. Over time, gait value converged toward 0.5 as muscle healing progressed as 0.52 (IQR, 0.48 to 0.54) in simple suture group, 0.52 (IQR, 0. to 0.51) in overlapping suture group and 0.51 (IQR, 0.47 to 0.57) in splitting suture group at Week 8 (Fig. 3).

However, we could not find statistical significance between all groups during the experimental period. This indicate that the suture technique was not influence the boundary of muscle contraction.

3.2. Simple suture demonstrates early remission of inflammation compared with overlapping and splitting sutures

In H&E staining, all groups demonstrates significant infiltration of inflammatory cells between muscle fibers at week 1. At week 8, overlapping and splitting groups still demonstrated marked infiltration of inflammatory cells to the interstitial tissue between muscle fiber. However, simple group demonstrates significant remission of inflammation compared with overlapping and splitting groups (Fig. 4).

IL-1β and TNF-α are cytokines which are important mediator of acute inflammatory response. At week 1, IL-1 expression are analogously high between muscle fibers in all groups. Although IL-1 expression has almost vanished in the simple and overlapping groups at week 8, the splitting group demonstrated still strong expression of IL-1 compared with the overlapping and simple groups. We could find similar chronological change in western blot for IL-1. Although IL-1 expression was similarly high in all groups at Week 1, simple group demonstrated decreased expression of IL-1 contrary to high expression of IL-1 in the overlapping and splitting groups at Week 8 (Fig. 5).

On immunohistochemistry and western blot, the overlapping and splitting suture groups demonstrated high expression of TNF-α compared with

the simple group at Week 1. At Week 8, simple group demonstrated clearance of TNF-α expression on immunohistochemistry and western blot. Although the overlapping and splitting groups demonstrated decreased TNF-α expression at Week 8, TNF-α expression was significantly higher than the simple group on immunohistochemistry and western blot (Fig. 6).

3.3. Simple and overlapping sutures demonstrate organized remodeling of muscle healing at week 8

In immunofluorescence staining for MyoD and Laminin, MyoD expressed in regenerative phase of muscle healing and Laminin is marker of mature skeletal muscle. At week 1, the expression of MyoD was similar high at muscle suture site in all groups. At Week 8, MyoD expression was significantly decreased in the simple group, contrary to remained expression of MyoD in the overlapping and splitting groups (Fig. 7) Regeneration phase of muscle wound healing was cessated in the simple group while the overlapping and splitting groups demonstrated prolonged muscle regeneration at Week 8. At week 1, the simple group demonstrated significant Laminin expression at muscle suture site compared with the overlapping and splitting groups. At Week 8. Simple group and overlapping groups demonstrated equivalent expression of Laminin at muscle suture site. However, the splitting group demonstrated scanty expression of Laminin at muscle suture site during experiment period (Fig. 8). This indicate that the splitting group has prolonged inflammation and regeneration phase followed by poor muscle

fiber remodeling.

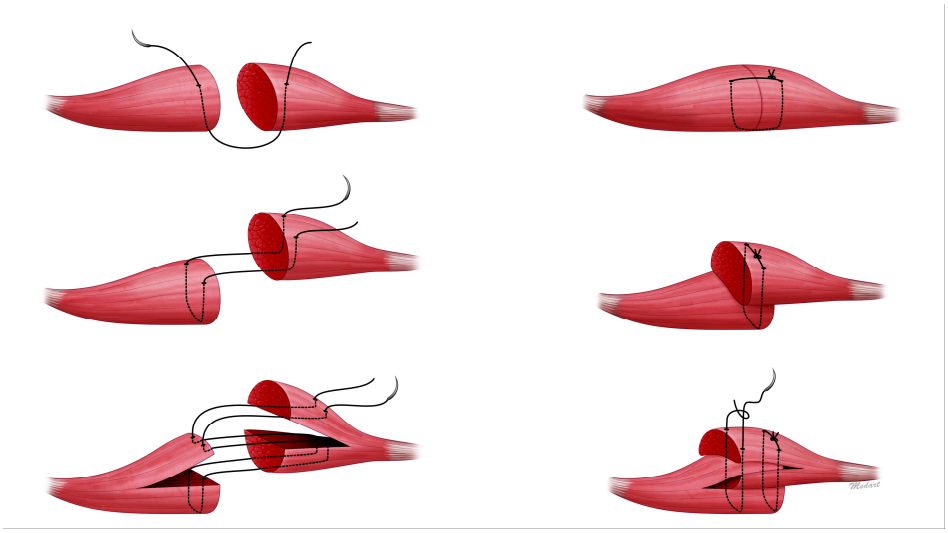


Figure 6. Muslce suture techniques (upper) Simple interrupted suture (middle) Overlapping suture (lower) Splitting-interdigitating suture

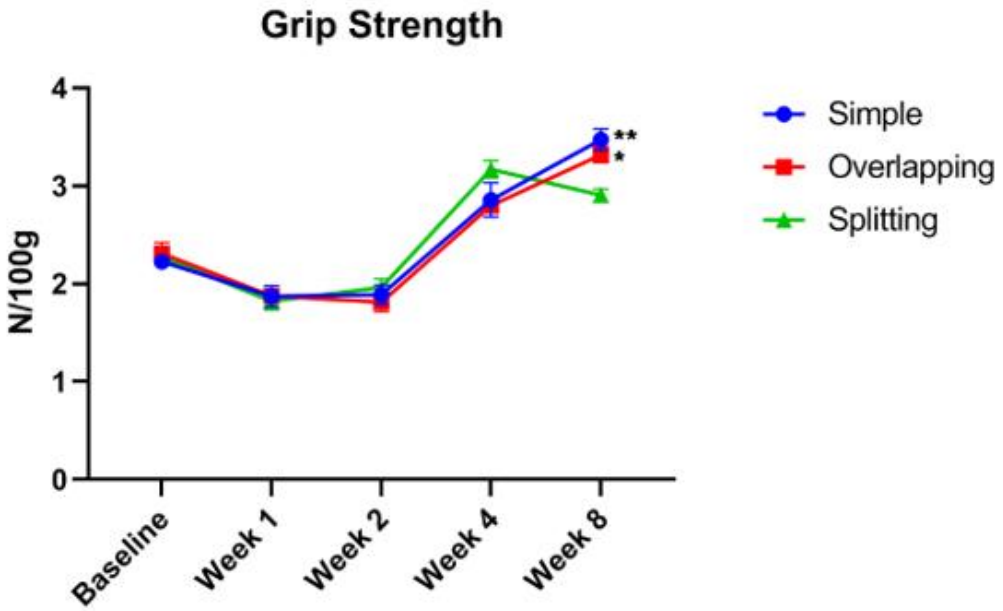


Figure 7. Grip strength test. Although all groups demonstrated same grip strength at baseline, simple interrupted suture and overlapping suture groups demonstrated significantly higher muscle power compared with splitting group at week 8 (simple vs splitting, p=0.0092; overlapping vs splitting, p=0.0113)

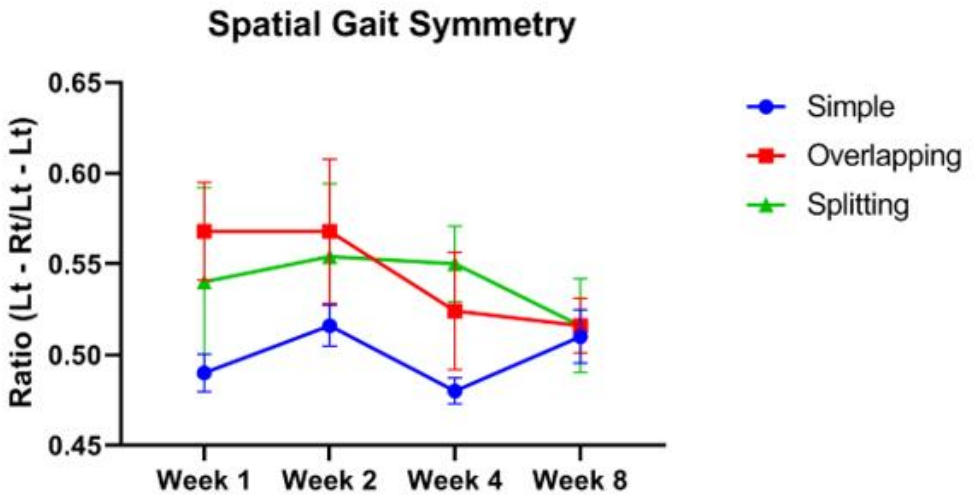


Figure 8. Gait test. Overlapping and splitting suture groups demonstrated asymmetric gait interval between left and right sides until through week 4. However, there was no statistical difference between all groups and gait difference was similar in all groups at week 8.

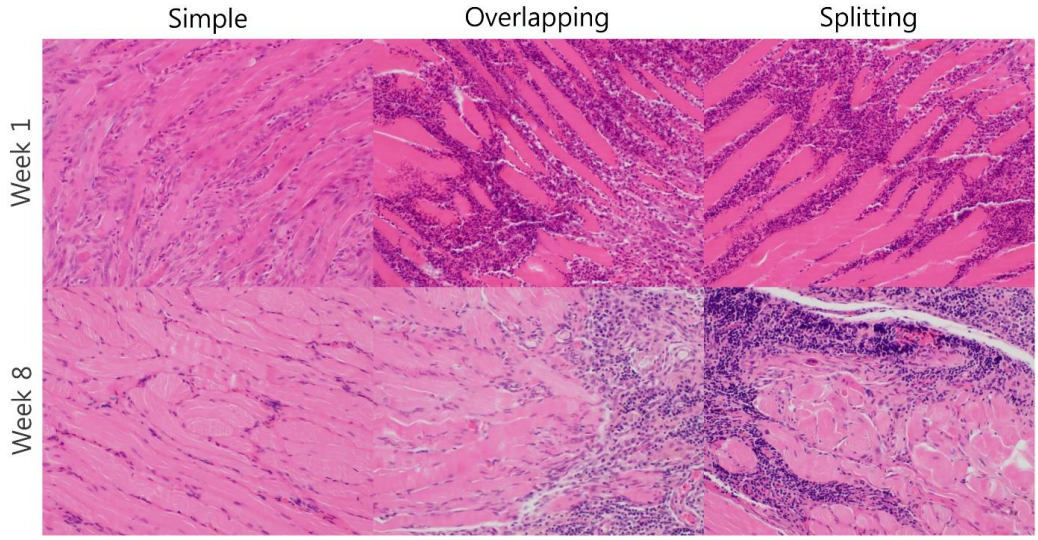
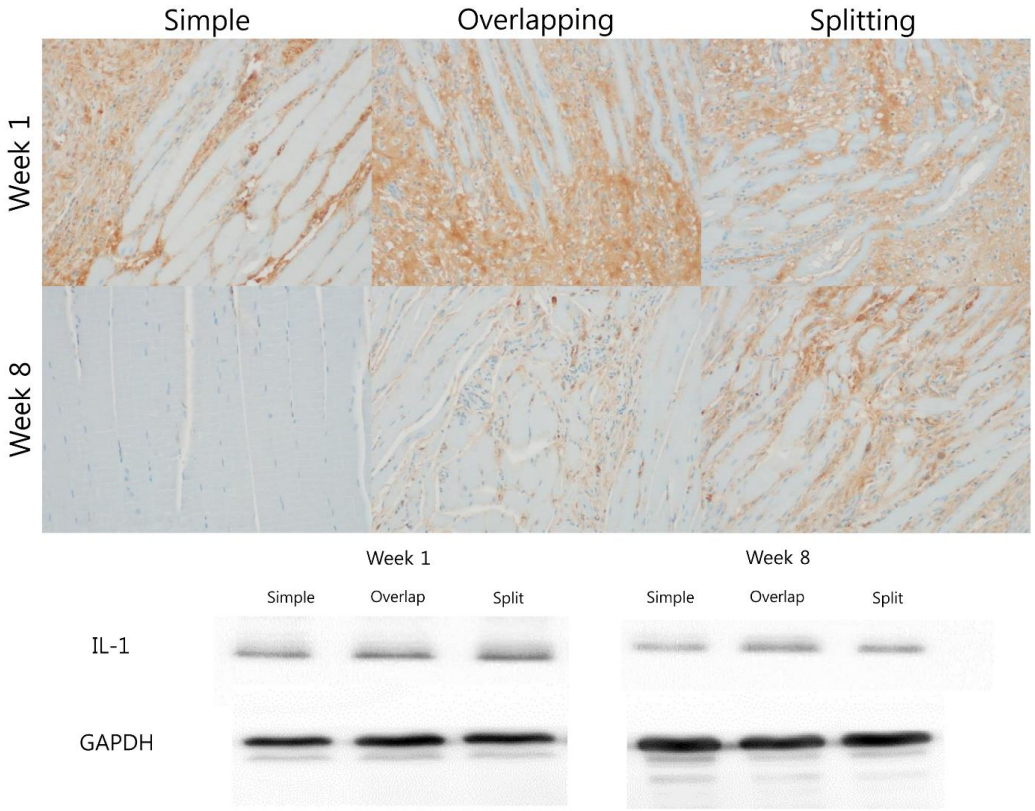


Figure 9. H&E staining. Overlapping and splitting groups demonstrated prolonged infiltration of inflammatory cells to interstitial tissue until week 8. In contrast, simple interrupted suture group was resolved inflammatory infiltration at week 8.



Figuer 10. Immunohistochemistry and western blot for IL-1. At week 1, all groups demonstrated strong expression of IL-1 on immunohistochemistry and western blot. Simple interrupted suture group demonstrated weaker expression of IL-1 compared with overlapping and splitting suture group on immunohistochemistry and western blot

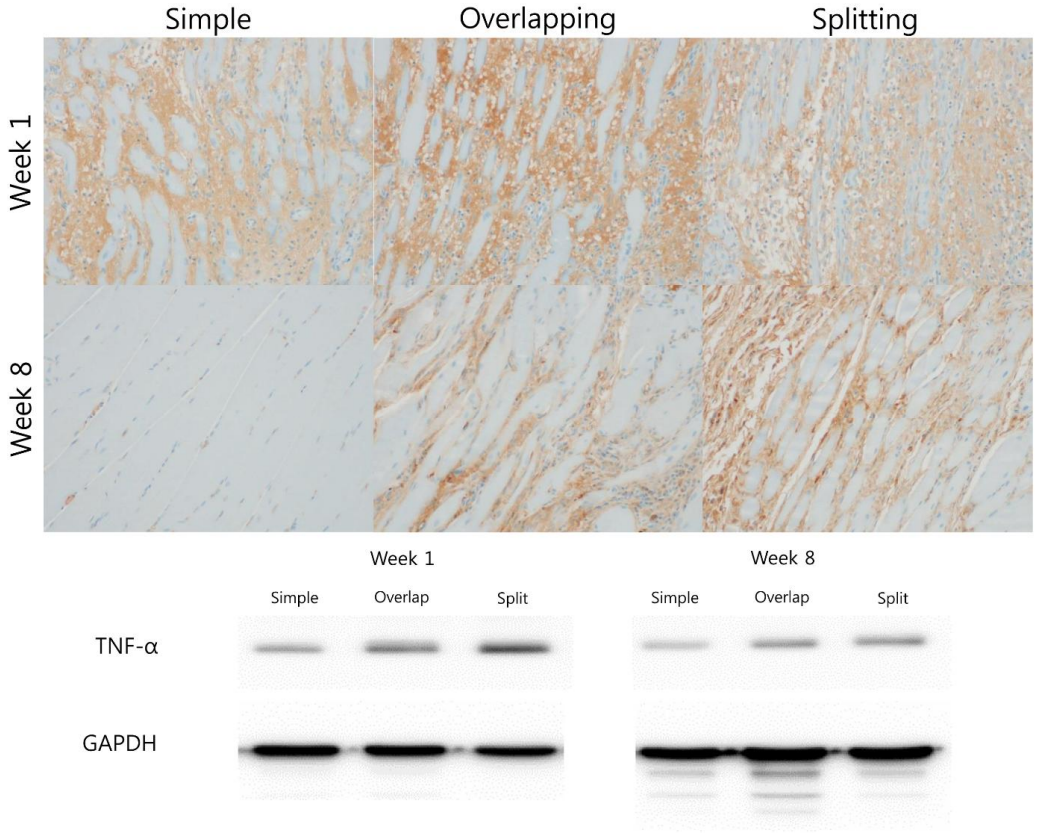


Figure 11. Immunohistochemistry and western blot for TNF-α. At week 1, all groups demonstrated strong expression of TNF-α at interstitial tissue on immunohistochemistry. Splitting suture groups demonstrated higher TNF-α level compared with overlapping and simple interrupted suture group at western blot. Simple interrupted suture group demonstrated weak expression of TNF-α compared with other groups on immunohistochemistry and western blot at week 8.

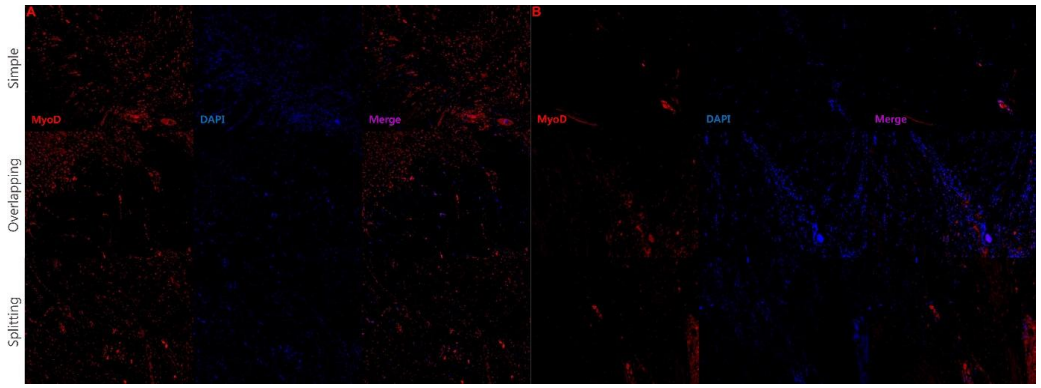


Figure 12. Immunofluorescence for MyoD. (A) Muscle regeneration was highly activated with strong expression of MyoD in all groups at Week 1. (B) Simple interrupted group decreased muscle regeneration activity with MyoD expression compared with overlapping and splitting suture group with retained expression of MyoD at week 8.

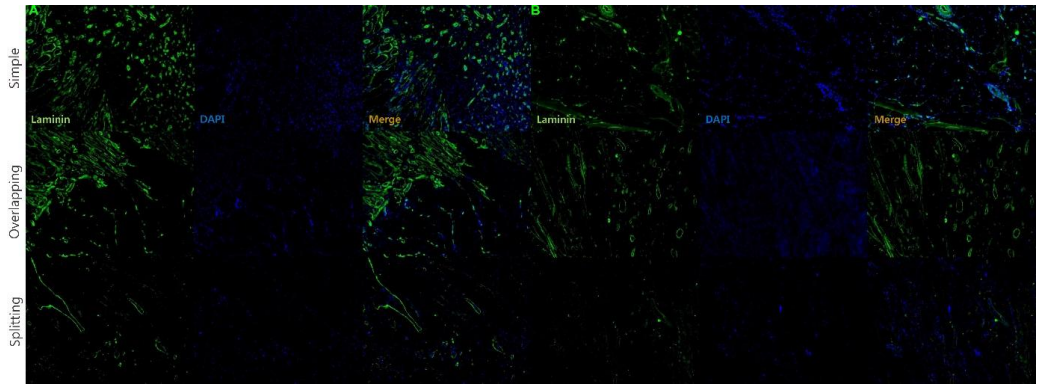
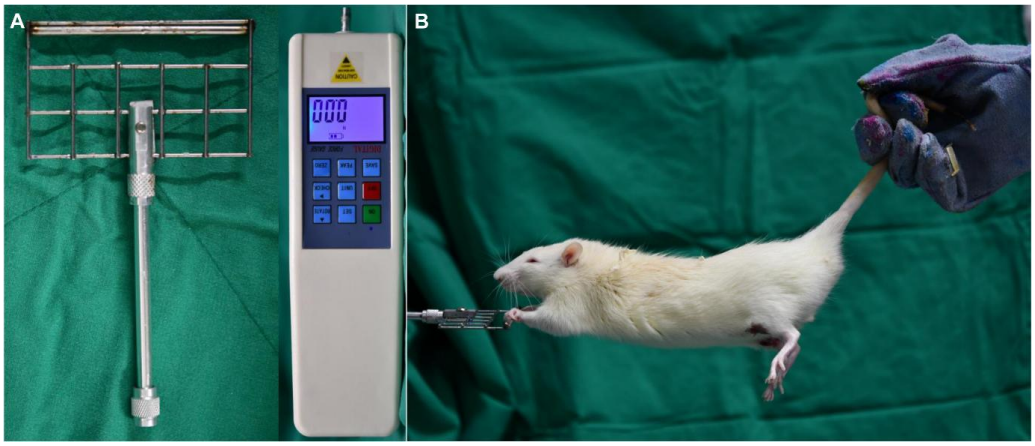
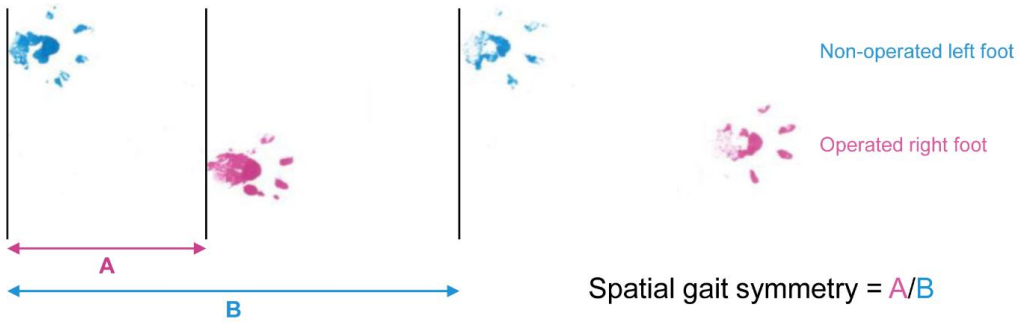


Figure 13. Immunofluorescence for Laminin. (A) Simple interrupted and overlapping suture groups demonstrated organized muscle remodeling with strong expression of Laminin at week 1 and 8. (B) In contrast, splitting suture group demonstrated weak expression of Laminin representing unorganized and scarce muscle remodeling until week 8.



Supplementary digital content 1. (A) Digital force transducer (B) The grip strength test was repeated twice and calculated as an average of measured values.



Supplementary digital content 2. Spatial gait symmetry test

4. Discussion

Our results show that simple suture and overlapping suture demonstrated more powerful muscle regeneration compared with splitting-interdigitating technique. However, there was no difference of range motion between all groups at Week 8 based on the result of gait test. Simple interrupted suture demonstrated weak inflammation compared with overlapping and splitting-interdigitating suture based on the result of H&E staining, immunohistochemistry and western blot. At 8 weeks, simple interrupted suture demonstrated resolution of regeneration phase with weak MyoD expression and start of remodeling phase with marked lamninin expression in contrast, the other groups stagnated in regeneration phase.

Muscle regeneration was progressed through time-dependent five phases; degeneration, inflammation, regeneration, remodeling, and functional recovery.[14] Injured muscle fiber efflux intracellular component triggering inflammatory reaction.[15] Neutrophil and mast cells were initially influx inflammatory cells to injured muscle. Degranulation of resident mast cells release pro-inflammatory cytokines, such as TNF-α and IL-1.[16] These cytokines recruit neutrophil to the lesion followed by phagocytosis of necrotic debris. Then, quiescent satellite cells of the basal lamina is activated and differentiated to myoblast which fused with existing myofiber and repair damaged muscle fiber.[14,17] Activated satellite cells expressed myogenic biomarkers such as Pax7, Mcad, VCAM1 and MyoD during regenerative phase.[18,19] The major structural protein of basal lamina, laminin is identified in mature skeletal muscle and networking with ECM.[20] Based on sequence of muscle wound healing, we analyzed the degree of TNF-α and IL-1 to determine

enhanced inflammation, MyoD to determine the degree of regeneration of muscle, laminin to determine muscle remodeling. Furthermore, we analyzed functional recovery based on muscle power and the range of motion.

In cleft lip repair, overlapping suture of OOM has be recognized for effective method of mimicking philtral column.[6] Chang et al.[6] reported overlapping mattress muscle suture demonstrated better philtral column on three-dimensional photographic anthropometry and ultrasonographic measurement compared with asymmetric edge-to-edge suture technique. However, muscle regeneration was conducted with differentiated myoblast fusing with existing myofibril. In overlapping suture, diastasis OOM was connected by side-to-side nature when cutting edge of muscle was not contact with opposite side of OOM muscle. On view of process of muscle healing, overlapping suture was unnatural technique that interrupt the step to fuse myoblast with intact myofibril. Impaired muscle healing result in inelastic scar tissue and unnatural morphology when muscle was contracted. Furthermore, it is difficult to guarantee that overlapped muscle will be maintained as initial morphology because muscle is continuously acting tissue. Actually, interrupted suture was demonstrated thick muscle regeneration and stronger resistance to tension compared with overlapping suture.[21] In concordance with previous study, our result was also demonstrated simple interrupted suture demonstrated strongest muscle power and less inflammatory reaction with better muscle regeneration in histological analysis. Overlapping suture demonstrated similar muscle power and the range of motion with increased inflammatory reaction. Indeed, the purpose of overlapping suture was the construction of thicker muscle to mimicking ridge of philtral column in cleft lip surgery and more powerful muscle with tightening of levetor veli muscle in palatoplsty. However, we could

not find beneficial effect of overlapping suture in the result of muscle power, the range of motion, and histological evaluation. On other hands, less inflammatory reaction in simple interrupted suture resulted in organized muscle remodeling on the H&E staining and the laminin immunofluorescence staining. Overlapping suture could not show favorable outcomes in functional and histologic results compared with simple interrupted suture. Furthermore, splitting-interdigitating suture technique demonstrated unfavorable results in muscle power, inflammatory reaction, and muscle regeneration. The majority of modification of OOM suture technique in cleft lip surgery was performed through splitting step of both or one-side OOM muscle, then interdigitating or folding or vertical mattress suture was performed.[7-9,22] Splitting procedure could induce additional muscle injury and inflammation that hinder the muscle regeneration. Therefore, muscle power was significantly decreased in splitting-interdigitating group compared with simple interrupted suture group in our study. We supposed that additional manipulation of muscle tissue could induce muscle injury, ischemia, and unfavorable regeneration.

One limitation of our study is that we used skeletal muscle than facial mimetic muscle. Fundamentally, the origin of satellite cells that is key cells for muscle regeneration is different between orofacial muscle and skeletal muscle. Satellite cells of orofacial muscle was originated from mesoderm while satellite cells of skeletal muscle from mesoderm or ectoerm.[23] Interestingly, soft palate muscle was regenerated with higher collagen deposition and few myofiber regeneration.[24] Furthermore, satellite cells from orofacial muscle demonstrated a delayed differentiation compared with those from skeletal muscle.[25] Orofacial muscle regeneration demonstrated more scar-forming healing than skeletal muscle. In other words, suture technique forming more scar

tissue in skeletal muscle could intensify a scar formation in orofacial muscle. Therefore, the regeneration of overlapping and splitting-interdigitating suture technique could be degenerated in orofacial muscle model. Although there was muscle injury model using orofacial model, majority were muscle defect model which could not apply suture technique and functional analysis.[24,26,27] Therefore, we necessarily used skeletal muscle model in this study. In future study, we would draw an uncontroversial result with a human study by imaging work-up or larger animal study using orofacial muscle.

5. Conclusion

In cleft lip/palate surgery, there were various suture technique for orbicularis oris muscle and levator veli palatine muscle. Overlapping suture and the modified suture technique including splitting technique were famous and well-known suture method to imitate philtral column or strengthen muscle power for cleft surgeons. However, simple interrupted suture was best muscle regeneration in histology and functional recovery compared with overlapping and splitting suture technique in this study. Therefore, we recommend to consider simple interrupted suture technique to enroll when muscle repair in cleft lip/palate surgery.

References

1. Lonic D, Morris DE, Lo LJ. Primary Overcorrection of the Unilateral Cleft Nasal Deformity: Quantifying the Results. *Ann Plast Surg* 2016;77 Suppl 1:S25-29
2. Kim S, Choi TH, Park JU, et al. Influence of modified Furlow double opposing Z-plasty on mandibular growth in Oriental patients with cleft palate and/or lip. *Ann Plast Surg* 2014;73:311-314
3. Wong LS, Lu TC, Hang DTD, et al. The Impact of Facial Growth in Unilateral Cleft Lip and Palate Treated With 2 Different Protocols. *Ann Plast Surg* 2020;84:541-544
4. Stal S, Brown RH, Higuera S, et al. Fifty years of the Millard rotation-advancement: looking back and moving forward. *Plast Reconstr Surg* 2009;123:1364-1377
5. Cutting CB, Dayan JH. Lip height and lip width after extended Mohler unilateral cleft lip repair. *Plast Reconstr Surg* 2003;111:17-23; discussion 24-16
6. Chang FC, Wallace CG, Hsiao YC, et al. Long-term comparison study of philtral ridge morphology with two different techniques of philtral reconstruction. *Int J Oral Maxillofac Surg* 2020
7. Kim S, Kwon J, Kwon GY, et al. Dynamic reconstruction of the philtrum using coronal muscle splitting technique in microform cleft lip. *J Craniofac Surg* 2014;25:742-745
8. Cho BC. New technique for correction of the microform cleft

lip using vertical interdigitation of the orbicularis oris muscle through the intraoral incision. *Plast Reconstr Surg* 2004;114:1032-1041

9. Li L, Xie F, Ma T, et al. Reconstruction of Philtrum Using Partial Splitting and Folding of Orbicularis Oris Muscle in Secondary Unilateral Cleft Lip. *Plast Reconstr Surg* 2015;136:1274-1278
10. Nguyen DC, Patel KB, Skolnick GB, et al. Progressive Tightening of the Levator Veli Palatini Muscle Improves Velopharyngeal Dysfunction in Early Outcomes of Primary Palatoplasty. *Plast Reconstr Surg* 2015;136:131-141
11. Huang H, Han Y, Akinade T, et al. Force balance reconstruction of the orbicularis oris in unilateral incomplete cleft lip. *J Plast Reconstr Aesthet Surg* 2020
12. Cho BC, Kim YH, Tian L, et al. Long-Term Outcomes of the Minimal Skin Incision Technique for Correcting Severe Microform and Minor-Form Cleft Lip With Philtrum Reconstruction Through the Intraoral Incision. *J Craniofac Surg* 2020;31:79-84
13. Bertelli JA, Mira JC. The grasping test: a simple behavioral method for objective quantitative assessment of peripheral nerve regeneration in the rat. *J Neurosci Methods* 1995;58:151-155
14. Forcina L, Cosentino M, Musarò A. Mechanisms Regulating Muscle Regeneration: Insights into the Interrelated and Time-Dependent Phases of Tissue Healing. *Cells* 2020;9
15. Yang Y, Jiang G, Zhang P, et al. Programmed cell death and its

role in inflammation. *Mil Med Res* 2015;2:12

16. Yang W, Hu P. Skeletal muscle regeneration is modulated by inflammation. *J Orthop Translat* 2018;13:25-32
17. Mauro A. Satellite cell of skeletal muscle fibers. *J Biophys Biochem Cytol* 1961;9:493-495
18. Scharner J, Zammit PS. The muscle satellite cell at 50: the formative years. *Skelet Muscle* 2011;1:28
19. Creuzet S, Lescaudron L, Li Z, et al. MyoD, myogenin, and desmin-nls-lacZ transgene emphasize the distinct patterns of satellite cell activation in growth and regeneration. *Exp Cell Res* 1998;243:241-253
20. Sanes JR. The basement membrane/basal lamina of skeletal muscle. *J Biol Chem* 2003;278:12601-12604
21. Jeong WS, Lee SS, Park EJ, et al. Comparison of Biomechanical and Histological Outcomes of Different Suture Techniques in Rat Rectus Abdominis Muscle Repair. *Ann Plast Surg* 2017;78:78-82
22. Fan Q, Li Y, Danning Z, et al. "Three-unit" muscle reconstruction in secondary cleft lip repair. *Cleft Palate Craniofac J* 2015;52:88-95
23. Harel I, Nathan E, Tirosh-Finkel L, et al. Distinct origins and genetic programs of head muscle satellite cells. *Dev Cell* 2009;16:822-832
24. Carvajal Monroy PL, Grefte S, Kuijpers-Jagtman AM, et al. A rat model for muscle regeneration in the soft palate. *PLoS One* 2013;8:e59193

25. Ono Y, Boldrin L, Knopp P, et al. Muscle satellite cells are a functionally heterogeneous population in both somite-derived and branchiomeric muscles. *Dev Biol* 2010;337:29-41

26. Pavlath GK, Thaloor D, Rando TA, et al. Heterogeneity among muscle precursor cells in adult skeletal muscles with differing regenerative capacities. *Dev Dyn* 1998;212:495-508

27. Carvajal Monroy PL, Grefte S, Kuijpers-Jagtman AM, et al. Fibrosis impairs the formation of new myofibers in the soft palate after injury. *Wound Repair Regen* 2015;23:866-873

Functional and Histologic Analysis of Popular Techniques for Muscle Suture in Cleft Lip and Palate

Jaehoon Kim

Department of Plastic and Reconstructive surgery
Graduate School

Keimyung University

(Supervised by Professor Woonhyeok Jeong)

(Abstract)

Various suture techniques have been used to repair divided muscle in cleft lip and palate. We performed animal study to evaluate which techniques have better outcomes in restoration of muscle.

30 adult male SD rats were used. Right triceps muscle was cut, then repaired with three different suture technique; simple (n=10), overlapping (n=10), and splitting group (n=10). Muscle was isolated from 5 rats for each group on post-operative week 1 and 8 respectively. H&E staining, western blot, immuno-histochemistry with anti TNF-alpha and IL-1beta antibodies, and immunofluorescence with anti-Laminin and anti-MyoD antibodies were performed to evaluate inflammatory response and regeneration of muscle fiber. Grip strength (N/100g), spatial gait were recorded before the surgery and post-operative week 1,2,4 and 8. At post-op week 8, grip force per weight of simple group (media, 3.49; IQR, 3.28-3.66) and overlapping group (median, 3.3; IQR, 3.17-3.47) were significantly strong compared with splitting group (median, 2.91;

IQR, 2.76-3.05). There was no statistical significance in range of motion between groups during the experimental period. Simple group demonstrates significant remission of inflammation in H&E staining, and less expression of TNF-alpha and IL-1beta in western blot and immunohistochemistry than other groups. In immunofluorescence, splitting group showed stronger expression of MyoD and weaker expression of Laminin than the others at week 8 indicating prolonged inflammation and regeneration phase followed by poor muscle fiber remodeling.

So, Simple interrupted suture is better option for histologic and functional recovery of muscle when compared to overlapping and splitting suture techniques.

구순구개열에서 근봉합 방법에 따른 근섬유 회복의 기능적, 조직학적 분석

김 재 훈
계명대학교 대학원
의학과 성형외과학 전공

(지도교수 정 운 혁)

(초록)

구순구개열에서 갈라진 근육을 복구하기 위해 다양한 방법이 사용되어 왔다. 우리는 흔히 사용되는 봉합 방법에 따른 근섬유 재생의 정도를 동물 실험에서 알아보았다.

30 마리의 SD 쥐가 사용되었다. 우측의 삼두근을 절개한 다음 단순 봉합 (10 마리), 오버랩 봉합 (10 마리), 스플릿 봉합 (10 마리) 방법으로 이어주었다. 수술 1 주, 8 주 후에 각각 그룹당 5 마리에서 조직을 채취하였다. 조직학적 검사는 H&E 염색, 웨스턴 블롯, 면역화학염색, 면역형광염색으로 이루어졌고 기능적 검사를 위해 악력을 측정하고 보행시 발자국을 기록하였다.

수술 후 8 주째 몸무게 100g 당 악력(N)은 단순 봉합 그룹에서 중앙값(median) 3.49 (IQR, 3.28-3.66), 오버랩 봉합 그룹에서 중앙값 3.3

(IQR, 3.17-3.47), 그리고 스플릿 봉합 그룹에서 중앙값 2.91 (IQR, 2.76-3.05)의 결과를 얻었다. 발자국 기록을 보았을 때 실험 기간 동안 모든 그룹에서 유의미한 관절 가동 범위의 차이는 없었다. 단순 봉합 그룹의 H&E 염색에서 염증 침윤 세포의 수가 현저히 낮았고 TNF-α, IL-1 항체를 이용한 면역화학염색에서도 낮은 수준의 발현을 보여주었다. 면역형광검사에서 스플릿 봉합 그룹은 수술 후 8주까지 강한 정도의 MyoD 와 Laminin 의 발현을 보여주었는데 이는 염증 반응의 지속과 지연된 근재생 및 재구성을 의미한다.

따라서 단순 봉합 방법이 근육의 조직학적 및 기능적 회복에 더 좋은 선택이 될 수 있다.

□ 저자 약력

1992년 대구 출생

계명대학교 의과대학 의학과 졸업

계명대학교 동산병원 인턴 수료

계명대학교 동산병원 성형외과 전공의(현)

계명대학교 대학원 의학과 석사과정 중