

Comparative Study of Inflammatory and Fibrotic Reactions Induced by Silicone Implants with Different Hardness

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Short Running Head: Experiment on Varied Silicone Hardness

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Abstract:

Background: Silicone nasal implants have been widely used in rhinoplasty. However, research on the correlation between implant hardness and the development of fibrosis and inflammation is lacking. This study investigated how the hardness of silicone implants influences fibrosis and inflammation through an experimental investigation.

Methods: Three types of nasal silicone implants with varying hardness levels were implanted bilaterally on the backs of 15 Wistar rats. Ten weeks post-implantation, clinical aspects, such as differences in immune reactions and formation of peri-implant capsules were analyzed. The intra-tissue response of the host was assessed by qRT-PCR using 11 markers. Specific markers were investigated by immunohistochemical (IHC) staining. Pathological grading was performed to investigate inflammation and fibrosis in the peri-implant tissues. Quantification and statistical analysis of these parameters were conducted to derive scores for intergroup comparisons.

Results: Histological analysis of the 45 capsules obtained revealed lower levels of fibrosis and acute inflammation in the group with lower hardness. This result was confirmed by IHC staining. Hematoxylin and eosin staining showed that the capsule thickness was less in the low-hardness group. Additionally, inflammation resolved quickly in the low-hardness group, and mature fibrotic tissue was observed.

Conclusions: When silicone implants are stably positioned within the body, less-rigid implants exhibit lower inflammatory or fibrotic responses. This is directly related to the adverse effects associated with capsule formation. These findings contribute to our understanding of the behavior of heterotopic materials in the nasal dorsum and their effects on the skin and soft tissue envelope.

Introduction

Silicone is a biomaterial valued for its thermal stability, chemical resistance, and low cost.¹ Its non-porous nature resists infection and allows easy sterilization, making it widely used in plastic surgeries.² In rhinoplasty, silicone implants are preferred over autologous grafts, especially in Asians with low nasal dorsum and short nose features.³

Silicone implants in rhinoplasty can cause chronic inflammation, infection, and capsular contracture (CC).⁴ CC is the formation of an abnormal fibrous capsule in response to foreign materials, primarily reported in augmented mammoplasty.⁵ In breast surgery, CC is mainly attributed to two causes: subclinical infection, where a slight infection occurs post-surgery causing inflammation and thickening of the capsule leading to CC and foreign body reaction, where the immune response to the implant results in inflammation and formation of a fibrous capsule that contracts over time.^{6, 7}

CC in rhinoplasty occurs through mechanisms similar to those in augmentation mammoplasty. The reported causes include inflammation and infection,⁸ excessive formation of fibrous capsule tissue, flawed surgical techniques such as over-resection of the cartilage, and anatomical characteristics of the nose.⁹ CC after augmentation rhinoplasty is mainly caused by silicone implants leading to external deformities, such as skin contraction, implant displacement, and nostril asymmetry, which can cause significant stress to the patient. This is because surgery involves not only correcting the shape, but also rebuilding and reconstructing the nasal framework.

This study investigated histological contracture reactions in the nose, focusing on inflammation, fibrosis, and capsule formation caused by implants with varying hardness. We hypothesized that different hardness levels of silicone implants elicit distinct inflammatory and

fibrotic responses. Our goal was to identify the potential for inflammation or capsule formation associated with varying implant types and hardness levels. By comparing tissue responses according to implant hardness, we hope to contribute to safer and more effective implant selection in clinical practice.

Material and Methods

Sample preparation

Nine-week-old Wistar rats were chosen for their suitability as an experimental model and the availability of baseline data.¹⁰⁻¹² The animals, divided into three groups by average body weight, received implants of varying hardness values:

- Material A (referred to as Group A): SOFTXiL[®] (BISTOOL Co., Seoul, Republic of Korea)
- Material B (referred to as Group B): SUN[®] (SUN MEDICAL Co., Uiwang, Republic of Korea)
- Material C (referred to as Group C): MISTI[®] (Keosan Trading Co., Seoul, Republic of Korea)

Implant hardness (23A, 41A, 45A) was measured using the Shore A scale; all shared a boat-shaped design. In this study, hardness was evaluated using the Shore A durometer scale, which provides a standardized measure of the material's resistance to deformation. All implants shared a boat-shaped design descending to the nasal tip. A description of the group composition is shown in Figure 1 and Table 1.

Group 1: five rats (Head: Group C/ Left: Group A/ Right: Group B)

Group 2: five rats (Head: Group B/ Left: Group C/ Right: Group A)

Group 3: five rats (head: Group A/ Left: Group B / Right: Group C)

Implant insertion and animal autopsy

This experiment was conducted in accordance with the ethical standards set by the Animal Ethics Committee, and the animals were humanely euthanized using appropriate methods. The experiment followed Woojung Bio's Ethics Committee regulations (IACUC2401-011). Three silicone implants were inserted at distinct locations in each rat, under the panniculus carnosus layer, mimicking the human superficial muscular aponeurotic system. Because the entire nasal implant could not be inserted into the rats, it was divided in half. (**See Figures, Supplemental Digital Content 1**, a silicone implant divided in half and inserted into the rat.) The implants, were placed in the head and left and right flanks (Fig. 1) and measured 3–4 cm along their long axis.

Rats were anesthetized with 2.5% isoflurane, incisions sutured post-implantation, and disinfected with povidone-iodine. Body weight and food intake were recorded twice After a 10-week period, the animals were euthanized for autopsy, with implants intact.

Histological Evaluation

The peri-implant capsule was well adhered to the implant, it was not tightly attached and could be easily separated from the implant. After separation, the capsule was rinsed with phosphate-buffered saline (PBS) and divided into two halves. One half of the capsule was fixed in formaldehyde and paraffin-embedded for histological analysis, while the other was stored in cryotubes for RNA extraction and qRT-PCR. For histological studies, including hematoxylin and eosin (H&E), Masson's trichrome (MT) staining, and immunohistochemistry (IHC), 6 μ m serial longitudinal sections were prepared. Capsule thickness was centrally measured to standardize data collection. H&E staining used a kit (SH-002, KPNT, Cheongju, Republic of Korea), while MT staining (MST-2-K, KPNT) assessed collagen deposition around the implants.

Measurements were consistently performed in intact capsule areas, avoiding regions with damage or deformation, to minimize bias and ensure reliable results. Slides were evaluated for fibrosis, inflammation, and small-vessel proliferation using a validated grading system from prior studies.¹³

Quantitative Real-Time Polymerase Chain Reaction

For RNA extraction, the remaining capsule was homogenized in 1 mL of TRIzol buffer in Eppendorf tubes, processed at 4°C for 1 minute (repeated five times), and centrifuged at 13,000 g for 10 minutes. The supernatant was transferred, mixed with 200 µL chloroform, left at room temperature for 3 minutes, then centrifuged. The RNA-containing supernatant was isolated, combined with 0.5 mL isopropanol at 4°C, and incubated on ice for 10 minutes. After a final centrifugation, the supernatant was discarded, and the RNA pellet was washed with 75% ethanol, dried, and dissolved in diethyl pyrocarbonate water.

RNA was transcribed into cDNA, and PCR quantified 11 wound-healing genes, including Collagen2A1, TNF- α , and TGF- β 2. Primer-probe sequences for the genes mentioned below are listed. (See Table, Supplemental Digital Content 2, which shows the sequence of the primers for qRT-PCR.)

Immunohistochemistry (IHC)

IHC staining was conducted on cryostat sections from frozen samples fixed in 4% paraformaldehyde. Endogenous peroxidase activity was blocked with 3% H₂O₂ in 100% methanol (1:5) for 30 minutes, followed by antigen retrieval in 0.02 M PBS. Sections were blocked with 5% blocking reagent at 37°C for 30 minutes, then incubated overnight at 4°C with primary antibodies against CD68 (ab31630, 1:100), TNF- α (ab1793, 1:100), and α -SMA

(ab5694, 1:1500) from Abcam, Cambridge, UK. Following PBS washes, secondary antibodies were applied for 30 minutes, and staining was visualized with 3,3'-diaminobenzidine chromogen.

Statistical analysis

The results were expressed as means with standard deviations. Group comparisons were performed using ANOVA (StatView 4.51, Abacus Concepts, Berkeley, CA, USA). When significant, Fisher's PLSD post-hoc test was applied. Additionally, ANOVA with Bonferroni correction was used. A significance level of 5% was applied.

Results

During the experimental period, no implants were extruded, and no rats expired.

H&E and MT staining results

The capsule thickness, measured quantitatively by H&E staining, was the thinnest in Group A (Fig. 2), showing statistically significant differences between groups when compared to Groups B and C (Fig. 3). MT staining, which was used to observe collagen tissue, was also performed. Capsule thickness was limited by severe adhesion, but Group A showed lower collagen deposition. (See Figures, Supplemental Digital Content 3, which shows the MT staining of group A, B, and C.)

qRT-PCR and IHC results

qRT-PCR was conducted using primers targeting specific gene markers for different cell types, allowing for the quantitative measurement of their presence and activity. For example, CD68 for macrophages, α -SMA for fibroblasts, and CD163 for giant cells are representative markers.¹⁴ Eleven immune-related markers were measured by qRT-PCR. Group A did not always show the lowest values for any of the markers. Group A had significantly lower markers for inflammation (IL-10, CD68), proliferation (α -SMA, vimentin), and remodeling (collagen

1A1) compared to other groups (Fig. 4 and Table 2). In summary, Group A generally exhibited lower expression of immune-related markers associated with inflammation and fibrosis than the other groups.

For the IHC analysis, marker expression was evaluated specifically in the capsule area. The expression of α -SMA was significantly higher in the capsule of Group A, indicating more mature fibrosis and active collagen laydown, while in Groups B and C, the expression of α -SMA in the capsule was minimal, and some individuals showed either immature or no fibrosis (Fig. 5). Additionally, for TNF- α , Group A showed slightly lower expression in the capsule, consistent with the qRT-PCR results (Fig. 6). The number of CD68-positive cells, a macrophage marker, was significantly lower in Group A compared to that in Groups B and C; thus, IHC confirmed that the inflammatory response in Group A was milder than that in Groups B and C. (**See Figures, Supplemental Digital Content 4**, which shows the IHC result for CD68.)

Pathologic grading system

Although Group A had a significantly thinner capsule, variations across sections could have affected the results, making the mean values alone insufficient. While PCR quantified tissue reactions, the grading system allowed qualitative comparison of tissue reactions between groups based on a pathologist's assessment. Histological findings included evaluation of acute and chronic inflammation, small vessel proliferation, and fibrosis formation (reflecting mature fibrosis).

Acute inflammation was not observed in Group A but was observed in three specimens in Group C (Fig. 7), suggesting less acute inflammation and more mature fibrosis in Group A (Table 3). Chronic inflammation was more pronounced in Groups B and C, with greater small vessel proliferation in granulation tissue. Fibrosis, marked by dense fibrotic tissue and collagen,

was mature in seven individuals in Group A, indicating a higher prevalence of mature fibrosis compared to other groups (Fig. 8 and **See Figures, Supplemental Digital Content 5**, which shows details of the H&E staining). Small-vessel proliferation was absent in Group A but present in Groups B and C. (**See Figures, Supplemental Digital Content 6**, which shows the small vessel proliferation in each group.) In summary, Group A showed weaker inflammation and more mature fibrosis.

Therefore, based on the histological findings, IHC and qRT-PCR results, Group A showed less foreign body reaction to the silicone implants, with a lower inflammatory response (inflammation phase) and more mature fibrosis (proliferation phase).

Discussion

During the proliferation phase of wound healing, new granulation tissue is formed around the implant which progresses, to form a fibrous capsule. In case of silicone implants, the fibrous capsule may harden during the remodeling phase, and in severe cases cause contracture. The remodeling phase typically involves tissue maturation and wound healing completion. However, with silicone implants, chronic inflammation may delay remodeling.¹⁵

The entire wound healing process requires a delicate balance between pro- and anti-inflammatory cytokines or chemokines. TNF- α and IL-10 are representative cytokines that fluctuate during the wound healing process. Low levels of IL-10 and high levels of TNF- α can be considered markers of an ongoing inflammatory response in the tissues.^{16, 17} TNF- α is a pro-inflammatory cytokine, while IL-10 suppresses inflammation. Reduced IL-10 expression suggests an immune attempt to control excessive inflammation.¹⁸ IL-10, on the other hand, functions to suppress inflammation, and a reduction in IL-10 expression around the implant suggests an attempt by the immune system to control excessive inflammation.¹⁹ In this study,

Group A showed lower levels of IL-10 and TNF- α compared to Groups B and C. CD68, a macrophage marker, was also significantly lower in Group A. This suggests that inflammation in Group A subsided more quickly, allowing faster progression to the fibrotic stage (proliferation phase).

Our results showed that TNF- α expression was lower in Group A and relatively higher in Groups B and C, which paralleled the results for CD68 expression, suggesting that Group A experienced a milder inflammatory response. The inflammatory and proliferative phases progressed rapidly in Group A, facilitating an earlier transition to the remodeling phase. α -SMA is also a crucial marker for granulation tissue, fibroblast activity, and the proliferation phase.²⁰ The qRT-PCR results in this study showed lower α -SMA levels in Group A compared to that in Groups B and C, which contrasts with the significantly higher α -SMA staining observed in Group A during IHC analysis. This discrepancy can be explained as follows:

First, qRT-PCR measures mRNA expression, whereas IHC detects protein expression, allowing for visual confirmation of the areas where α -SMA is locally expressed within cells.^{21, 22} mRNA expression does not always correlate directly with protein expression. In this study, even though α -SMA mRNA levels were low in Group A, the higher stability of the expressed protein may have resulted in increased α -SMA staining observed through IHC.²³ Therefore, the results suggest that in Group A, α -SMA protein accumulated specifically in the capsule region of the more stable and mature fibrotic capsule, with the most active collagen laydown occurring in Group A as observed in the IHC results. However, overall α -SMA mRNA expression, as indicated by the qRT-PCR results, was the lowest in Group A.

Additionally, upon reviewing the IHC and H&E slides with a pathologist, we observed that the granulation tissue in Group A exhibited small vessel proliferation surrounded by smooth muscle cells expressing α -SMA (Fig. 2 and Fig. 5). Considering these morphological findings alongside the α -SMA results, the lower α -SMA levels in Group A from the qRT-PCR could be interpreted as a consequence of the disappearance of granulation tissue around the implant capsule rather than a sign of ineffective fibrosis progression. Because granulation tissue forms primarily during the remodeling phase of wound healing, a lower amount of granulation tissue may indicate that Group A underwent a less pronounced remodeling phase.

To evaluate the remodeling phase of wound healing, which is closely related to capsule formation, specific methods can be used, such as visual assessment of collagen accumulation and rearrangement using MT staining.²⁴ Collagen PCR markers can also be used to quantify the presence of collagen in tissues, or the expression of TGF- β , a key regulator in the remodeling phase, can be analyzed.²⁵ Additionally, myofibroblast presence in the tissue, which is associated with wound contraction and extracellular matrix (ECM) restructuring, can be confirmed by α -SMA staining.^{26, 27}

Based on this study, two possibilities can be considered regarding the results showing lower expression of IL-10, CD68, α -SMA, Vimentin, and Collagen 1A1 in Group A. The first possibility is that Group A experienced fewer inflammation, proliferation, and remodeling phases. Characteristic molecular markers from both the RT-PCR and IHC analyses were expressed at low levels across the board, indicating that the overall tissue response to the implant was less pronounced. The second possibility is that Group A underwent a rapid transition from the inflammatory to remodeling phase due to the short duration of inflammation and

proliferation, resulting in quick stabilization of the tissue response. Group A's thinner capsule, supports this hypothesis.

Considering that this experiment was conducted over 10 weeks, which is sufficient time for wound healing to progress into the remodeling phase in Wistar rats,²⁸ the results suggest that the inflammation and proliferation phases in Group A were short-lived and that the tissue rapidly entered the remodeling phase. This can be interpreted as quick stabilization of the tissue response.

Hardness refers to a material's resistance to deformation; harder implants are less likely to deform under external forces.²⁹ Mechanical stress refers to the pressure or friction exerted by an implant on surrounding tissue. Harder implants resist force more, potentially increasing pressure or friction on nearby tissue. Increased hardness of a silicone implant within tissue can elevate mechanical stimulation. Particularly in thin-skinned areas like the nose with abundant soft tissues such as cartilage, harder implants can cause mechanical irritation to the nasal envelope.³⁰ Sunwoo et al.⁹ suggested that mechanical stimulation from silicone implants in capsules removed due to contracture induces chronic nerve inflammation, leading to fibroblast and mast cell activation, and CC formation. This aligns with studies showing excessive mechanical stimulation induces α -SMA activation, leading to collagen overproduction and ECM contraction, ultimately causing contracted nose.³¹ Findings from our study indicate that increased hardness correlates with higher α -SMA, CD68, and TNF- α expression, experimentally supporting that chronic mechanical stimulation promotes fibrotic responses.

This study's limitations include using a rat model, which may not fully represent human responses. However, Wistar rats were chosen for their shorter life cycle and rapid biological processes, making them a suitable model for studying capsule formation. Previous studies have

demonstrated that an 8–10-week observation period is sufficient for evaluating capsule formation and remodeling in Wistar rats.³² Nevertheless, future research using human tissues is required to validate these findings in clinical settings. Additionally, the study utilized 45 tissue samples from 15 rats, which may have limited the statistical power to detect significant differences across groups. For instance, no significant differences were observed in the pathological grading system across categories, possibly due to insufficient samples to compare histological differences. Future studies with larger sample sizes and extended observation periods in human models are warranted to further substantiate these findings.

Furthermore, the study primarily focused on Shore A durometer as the key mechanical property to evaluate the relationship between implant hardness and biological responses, such as fibrosis and inflammation. While this property is a critical determinant of material flexibility and mechanical resistance, other parameters, such as compressive strength, porosity, and modulus of elasticity, were not evaluated. These properties, along with biomechanical factors like lateral wall compression, elasticity, and shear stress, are important for understanding the broader performance of nasal implants. Future studies should incorporate these parameters to provide a more comprehensive analysis of implant behavior and its influence on tissue responses.

In addition, soft implants may be more suitable for nasal implants due to constant friction with cartilaginous structures, whereas chin implants, which are firmly attached to the periosteum, experience less dynamic interaction. As a result, harder implants with higher Shore A durometer are commonly used for the chin. Future studies comparing tissue responses of chin implants could provide broader insights into optimizing implant materials for different facial regions.

Conclusion

In this study, we found that in the group with lower hardness, inflammation resolved quickly, and proliferation transitioned efficiently, leading to an early remodeling phase transition. This suggests that chronic mechanical stress from increased implant hardness significantly enhances fibrotic responses and contributes to contracture development. Softer implants reduced tissue irritation, contributing to more natural outcomes.

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Figure legends

Figure 1. A schematic representation of implantation of silicone implants in 15 Wistar rats. "A," "B," and "C" denote implants with different hardness levels.

Figure 2. H&E staining of the capsules measured in each group. In groups A (A) it is thin, and in Group B (B), and Group C (C) it is relatively thick.

Figure 3. Comparison of capsule thickness between Groups A, B, and C (* <0.05, ** <0.01, *** <0.001).

Figure 4. qRT-PCR analysis of immune-related markers. Group A showed significantly lower expression of markers for inflammation (IL-10, CD68), proliferation (α -SMA, vimentin), and remodeling (collagen 1A1) compared to Groups B and C (* <0.05, ** <0.01, *** <0.001).

Figure 5. IHC result α -SMA in the capsule area. Group A had higher α -SMA expression, indicating mature fibrosis, while Groups B and C showed minimal or no fibrosis.

Figure 6. IHC result for TNF- α in the capsule area. Group A had slightly lower TNF- α expression than Groups B and C.

Figure 7. This is a representative slide that evaluates the degree of acute inflammatory cell infiltration. There is no neutrophil infiltration, which is an acute inflammatory cell, in Group A (A), but neutrophilic infiltration is observed in some cases in Group C (B).

Figure 8. The slides histologically evaluated for collagen formation with MT staining show that sparse collagen deposition in Group A (A), while thick collagen in Groups B (B) and dense collagen deposition in Group C (C) compared to that in Group A.

SDC legend

Figure, Supplementary Digital Content 1: The silicone implants were divided equally into half in all groups (A) and properly inserted into the panniculus carnosus layer of the rats (B).

Table, Supplementary Digital Content 2. A Table of sequence of the primers for qRT-PCR

Figure, Supplementary Digital Content 3: In MT staining, collagen accumulation in group A (A) was observed to be significantly the lowest in most samples.

Figure, Supplementary Digital Content 4: IHC result for CD68 in the capsule area. Group A (A) had significantly fewer CD68-positive cells compared to Group B (B) and Group C (C).

Figure, Supplementary Digital Content 5: This figure shows representative slides from Groups A, B, and C, evaluated for fibrosis formation using HE staining. A thin fibrotic wall is observed in Group A (A), a thick fibrotic wall in Group B (B), and a dense fibrotic wall in Group C (C).

Figure, Supplementary Digital Content 6: In group A (A), small vessel proliferation is not observed, while in groups B (B) and C (C), small vessel proliferation is observed in granulation tissue

Table 1. Composition of the experimental group

Group	Shore A scale	Implant site (Head/Left/Right)	Number of Animals
G1	23A	C/A/B	5
G2	41A	B/C/A	5
G3	45A	A/B/C	5

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Table 2. RT-PCR

	A	B	C	SD			P	post hoc test^a
Collagen2A1	1.236	1.036	1.375	0.959	1.037	1.018	0.652	-
TNF- α	3.039	4.062	5.725	5.379	4.677	6.467	0.417	-
TGF- β 2	1.453	0.361	1.584	1.468	0.519	1.499	0.019	-
IFN- γ	1.317	1.670	1.607	1.043	1.966	1.449	0.799	-
IL-4	1.094	0.801	1.293	0.470	0.713	0.746	0.129	-
IL-6	1.297	0.782	2.393	1.038	1.030	1.850	0.008	-
IL-10	2.063	3.405	7.957	2.492	2.648	8.410	0.010	A < C
CD68	1.228	2.049	10.349	0.944	1.368	5.636	<0.001	A, B < C
α -SMA	1.179	2.591	3.507	0.756	2.213	2.237	0.005	A < C
Vimentin	1.859	5.082	1.033	2.022	3.312	0.786	<0.001	A, C < B
Collagen1A1	1.322	3.271	1.392	0.909	2.176	1.082	0.001	A, C < B

^a post hoc tests: We conducted an analysis of variance (ANOVA) and performed post hoc tests

using the Bonferroni correction method.

Table 3. Pathologic semiquantitative scoring of peri-implant tissue reaction

	AG (n = 15)	BG (n = 15)	CG (n = 15)	P
Fibrosis				
Mature fibrosis	7 (46.67)	4 (26.67)	2 (13.33)	0.102
Immature fibrosis	8 (53.33)	9 (60.00)	13 (86.67)	
No fibrosis	0 (0.00)	2 (13.33)	0 (0.00)	
Acute inflammation				
Absent	15 (100.00)	15 (100.00)	12 (80.00)	0.096
Present	0 (0.00)	0 (0.00)	3 (20.00)	
Chronic inflammation				
Absent	1 (6.67)	0 (0.00)	0 (0.00)	0.496
Mild	6 (40.00)	9 (60.00)	10 (66.67)	
Moderate	6 (40.00)	6 (40.00)	4 (26.67)	
Severe	2 (13.33)	0 (0.00)	1 (6.67)	
Small vessel proliferation				
Absent	9 (60.00)	7 (46.67)	7 (46.67)	0.701
Present	6 (40.00)	8 (53.33)	8 (53.33)	

Figure 1

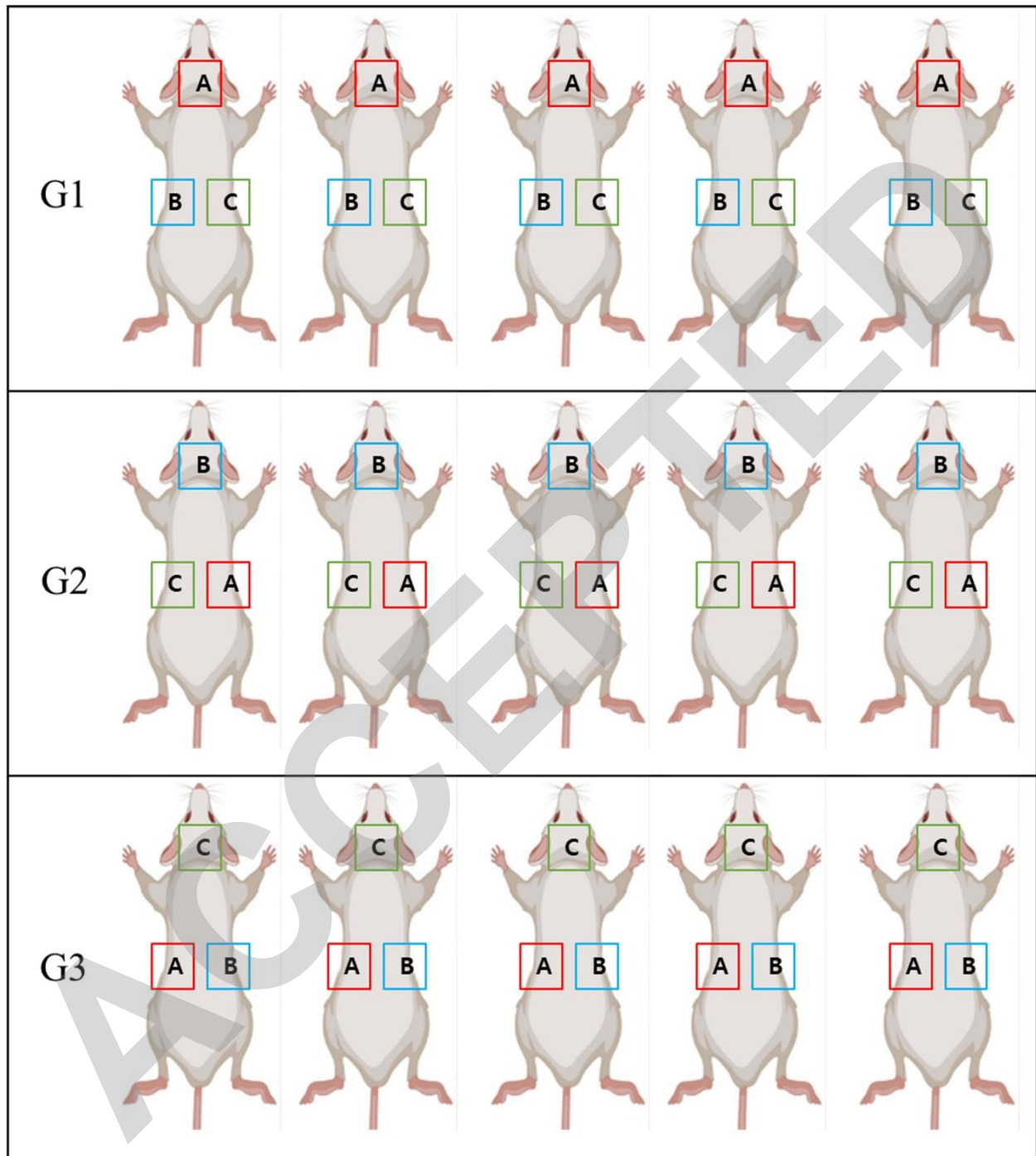


Figure 2A

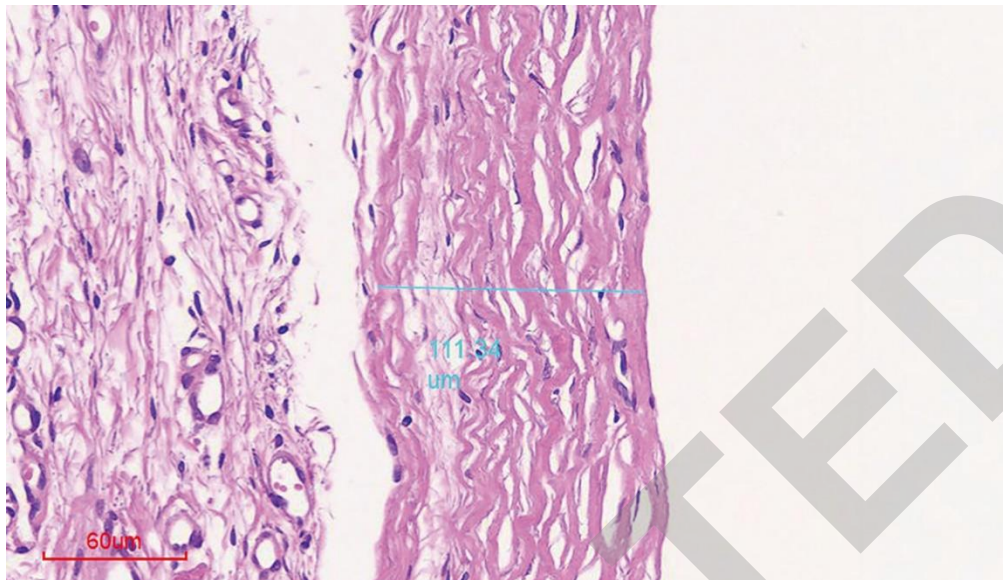


Figure 2B

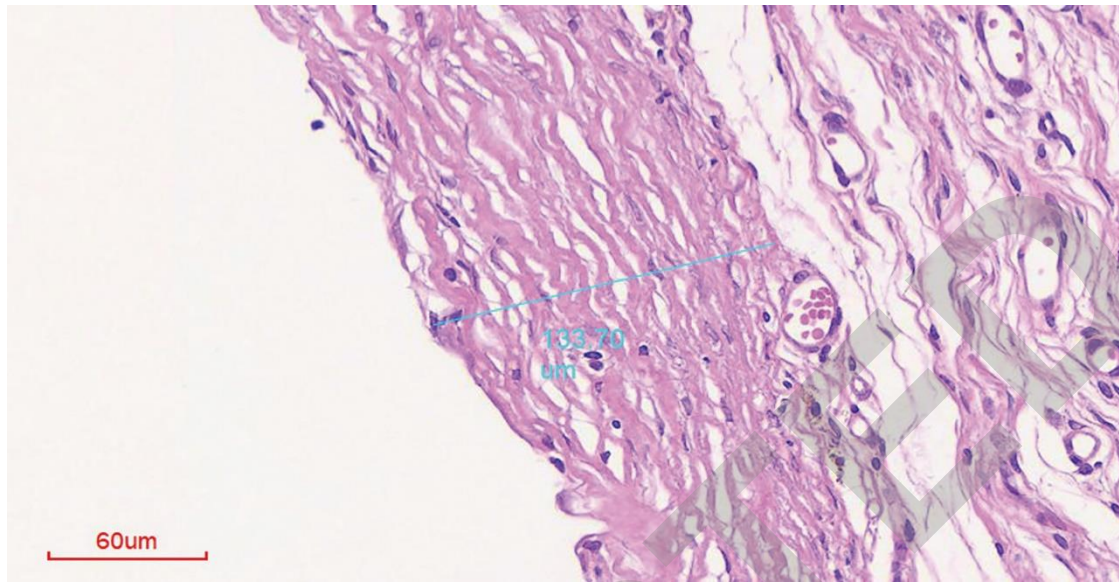


Figure 2C

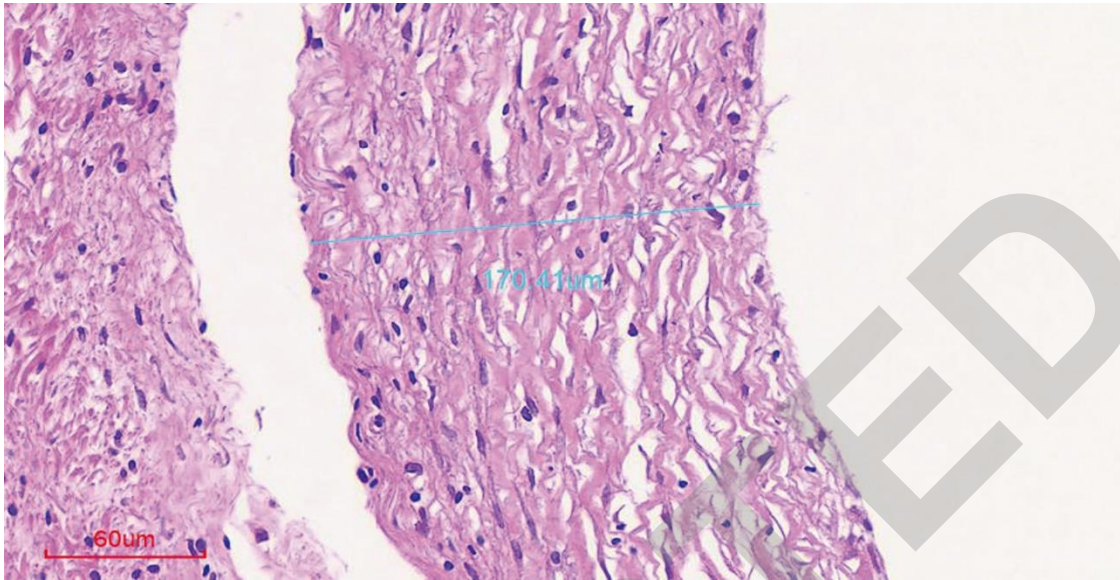


Figure 3

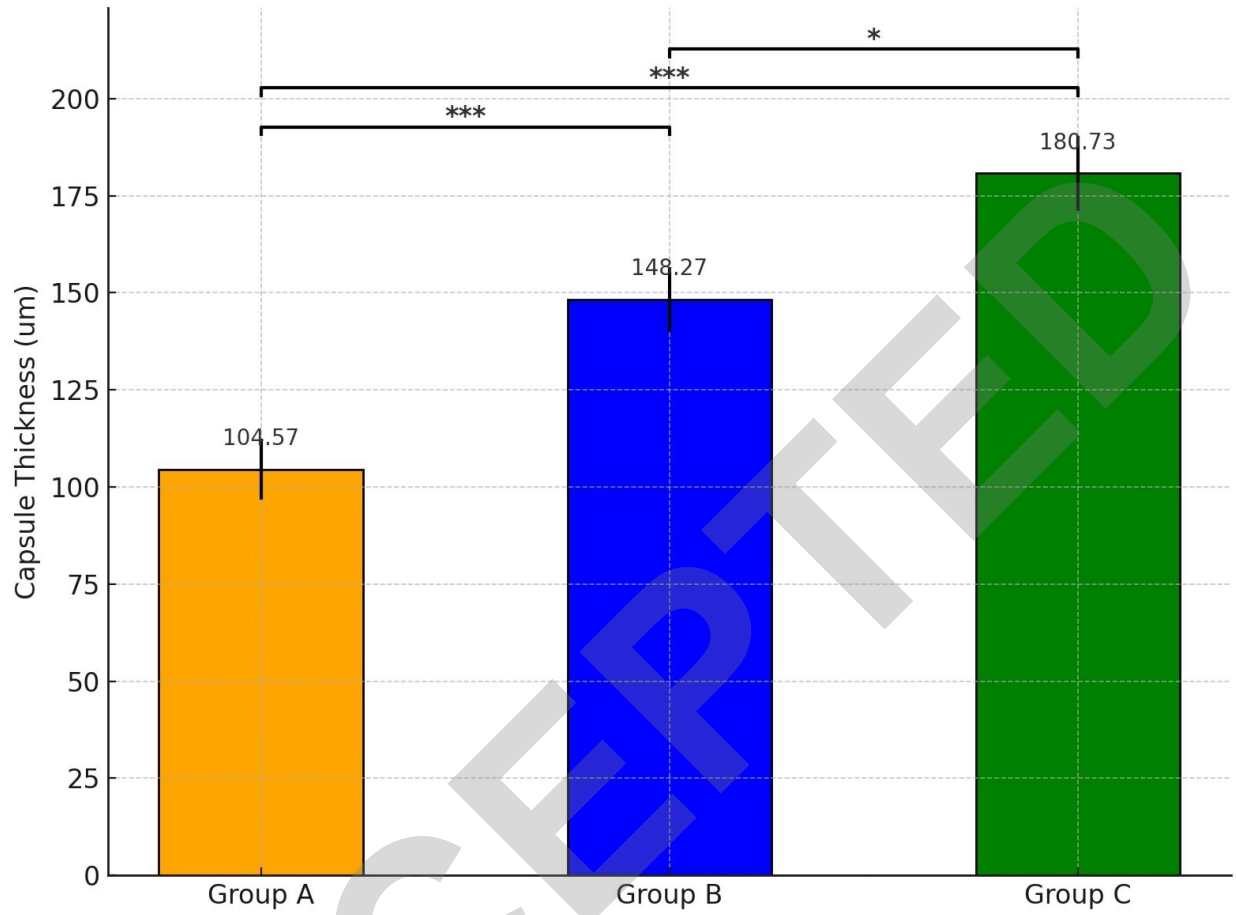


Figure 4

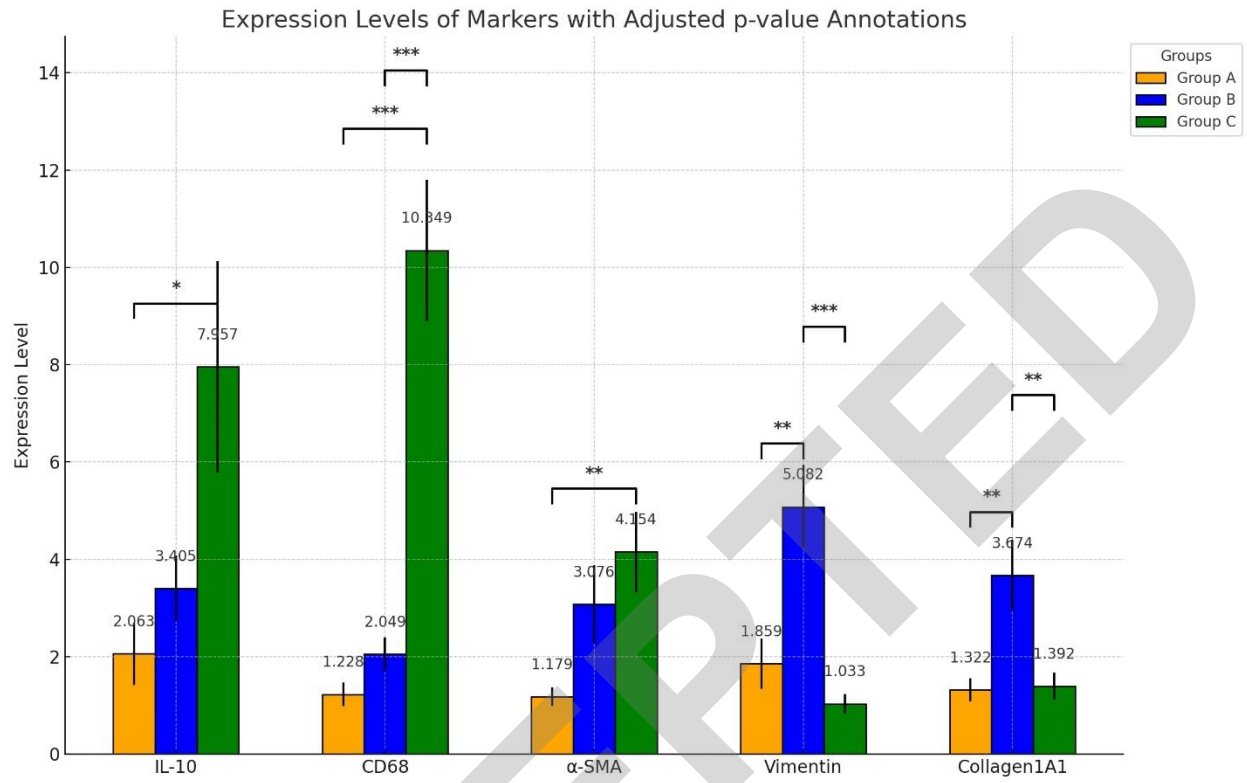


Figure 5A

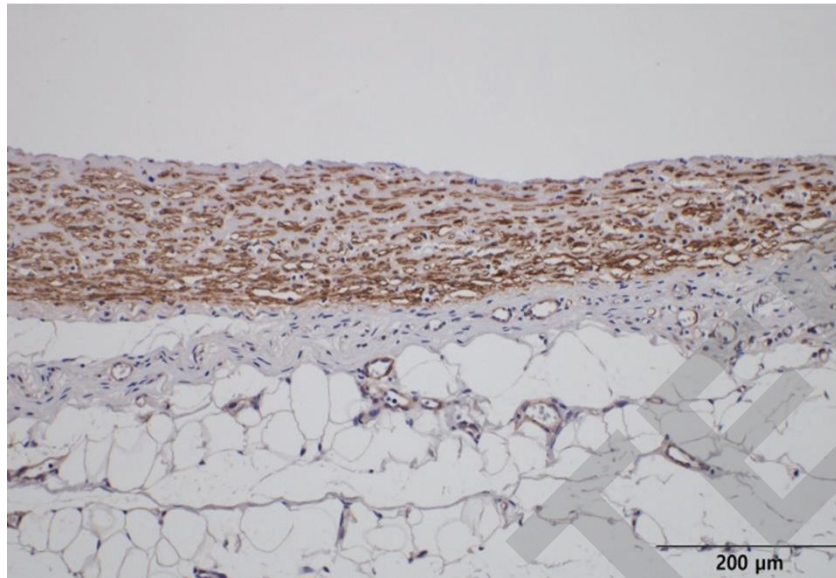


Figure 5B

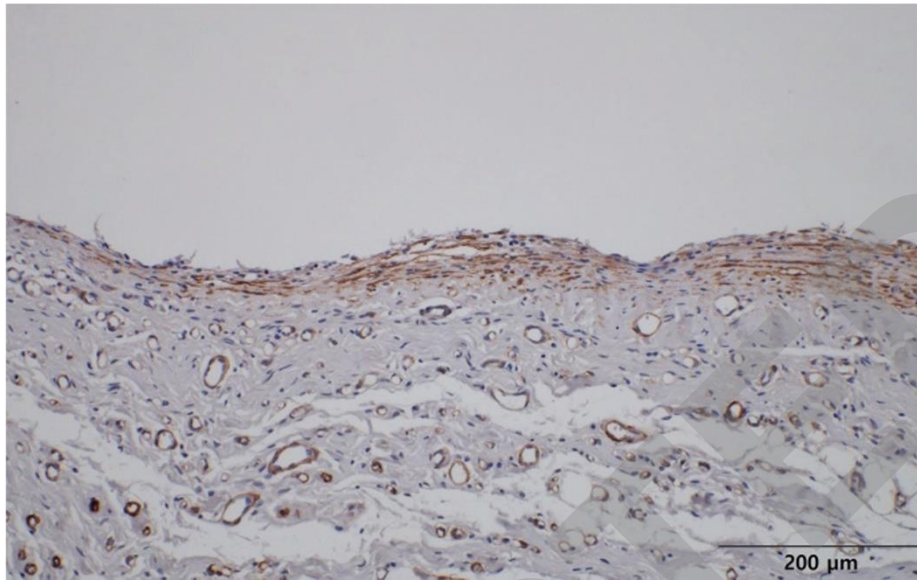


Figure 5C

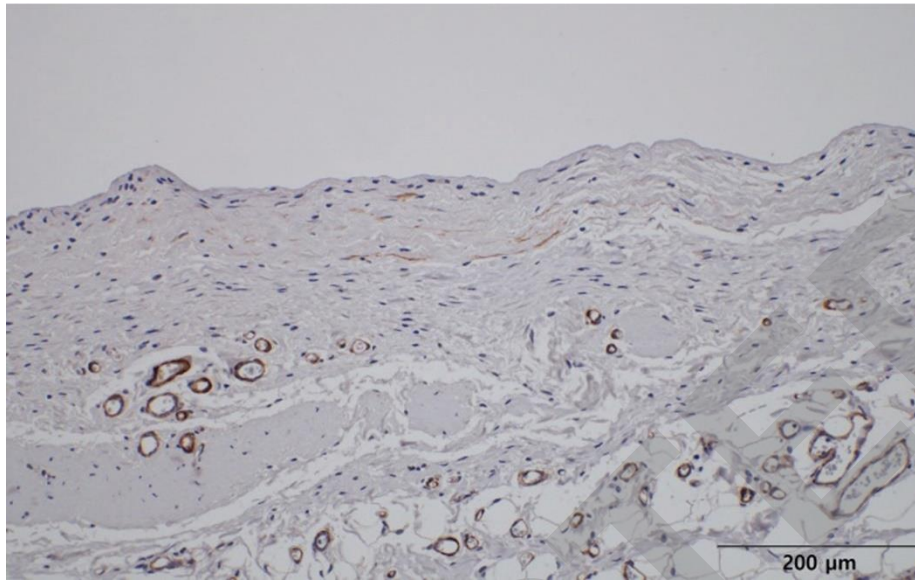


Figure 6A

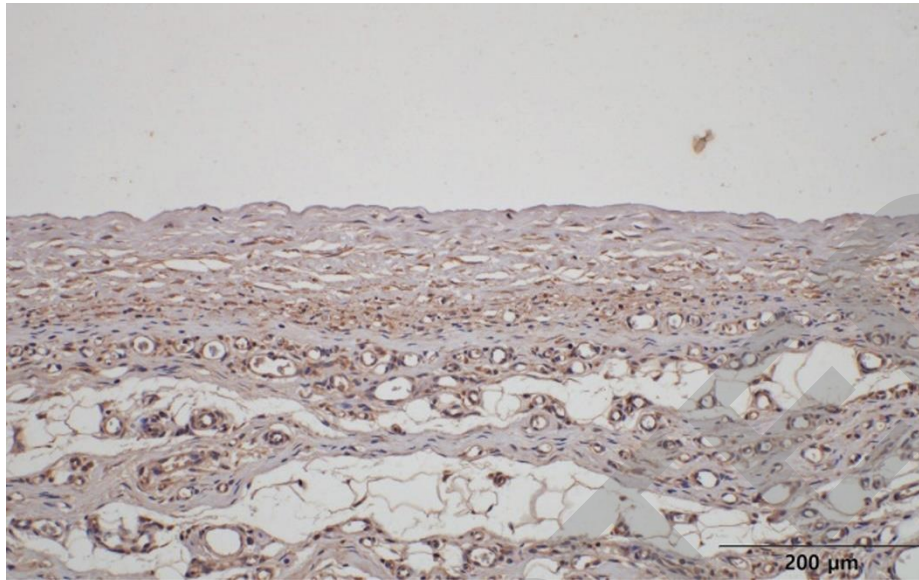


Figure 6B

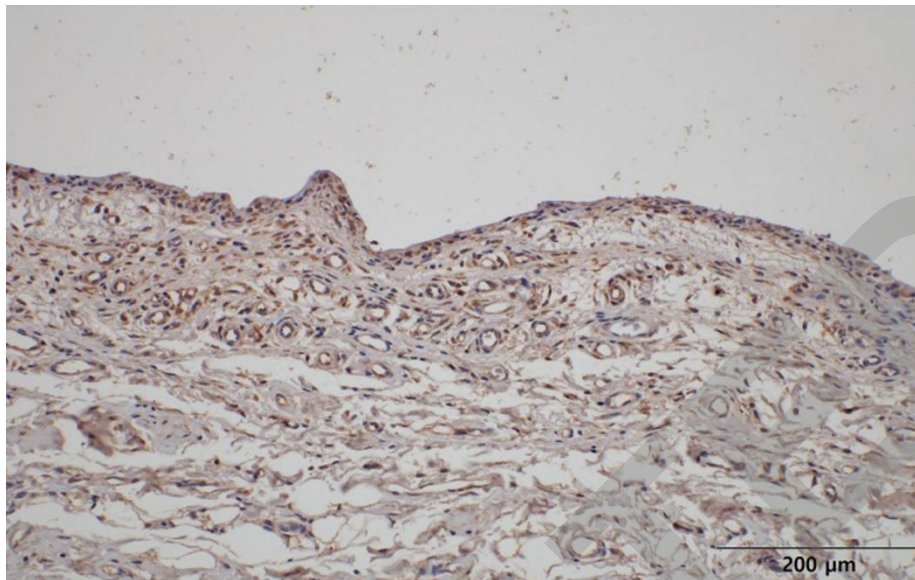


Figure 6C

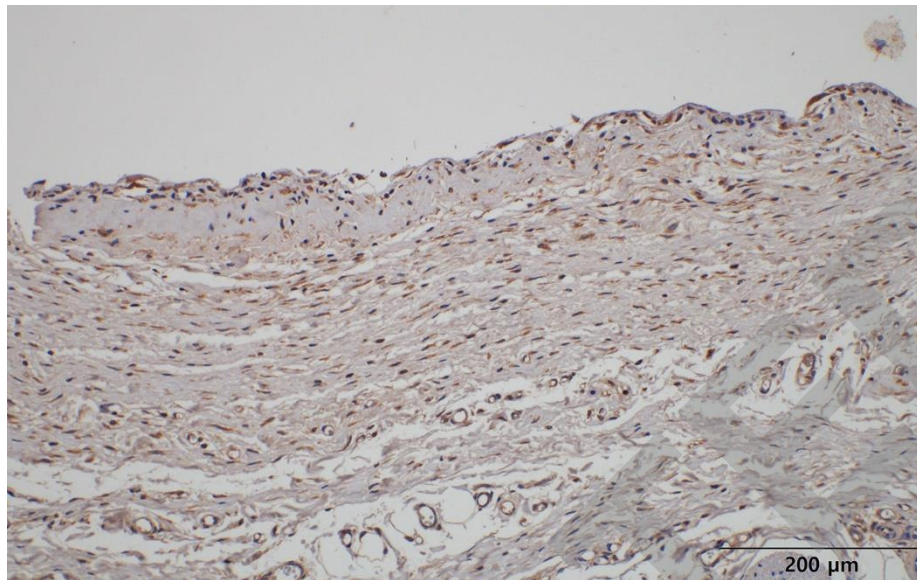


Figure 7A

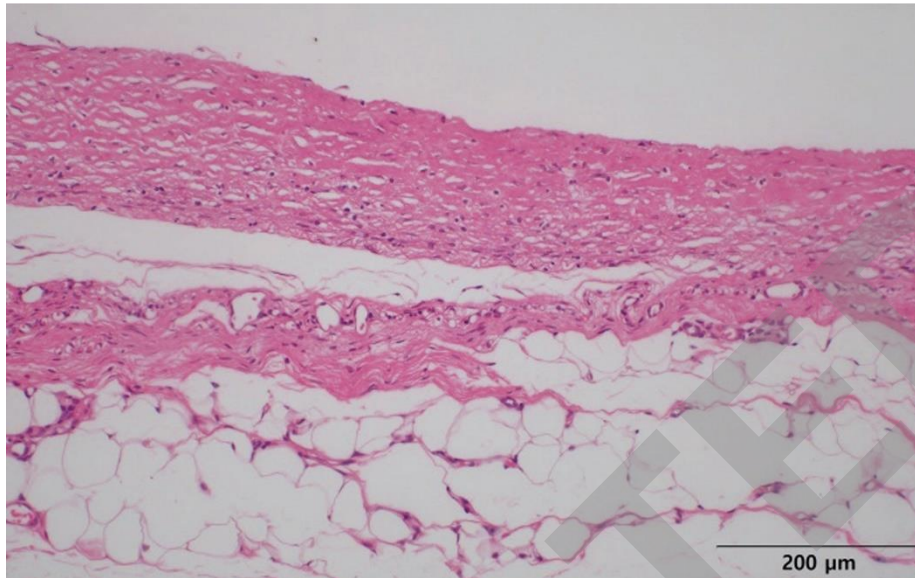


Figure 7B

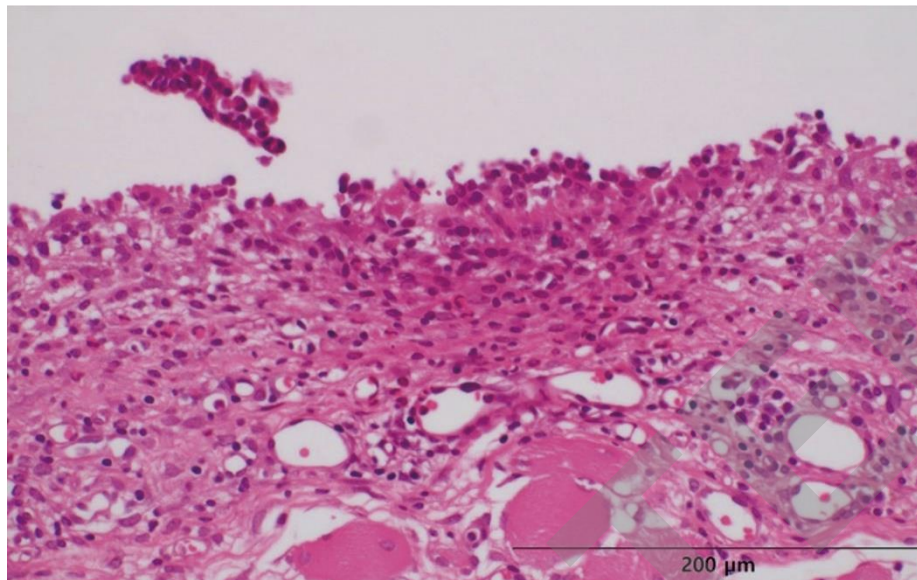


Figure 8A

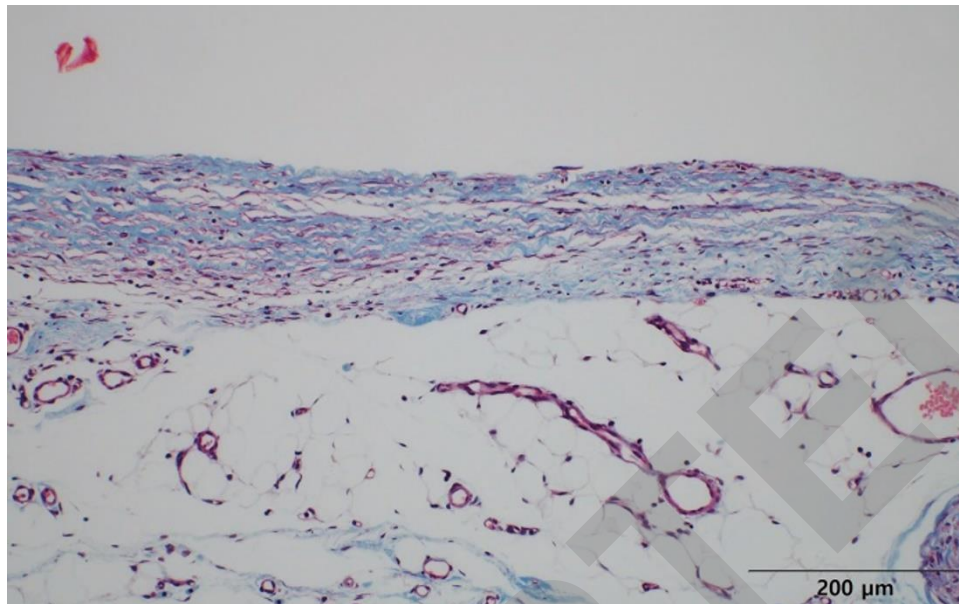


Figure 8B

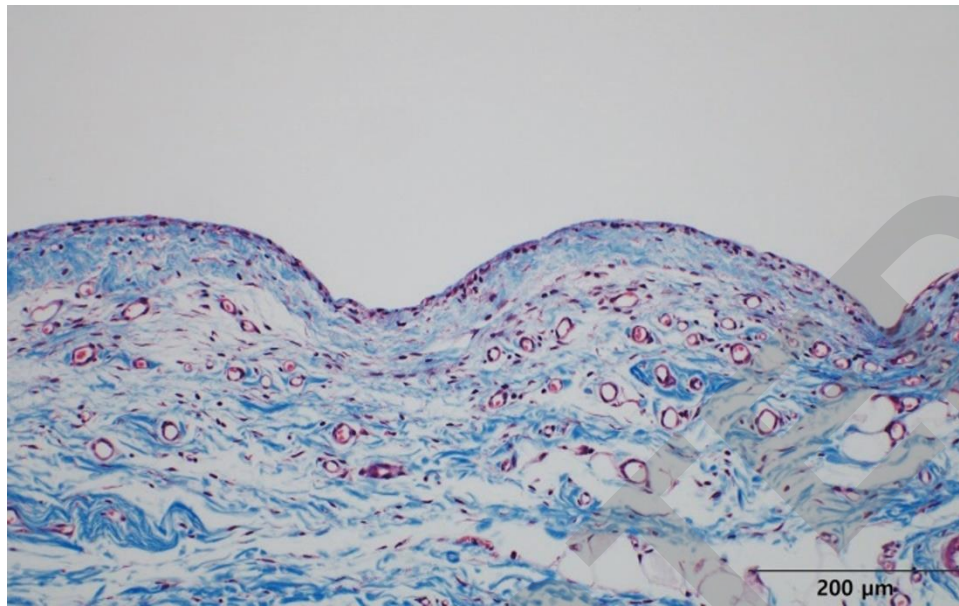
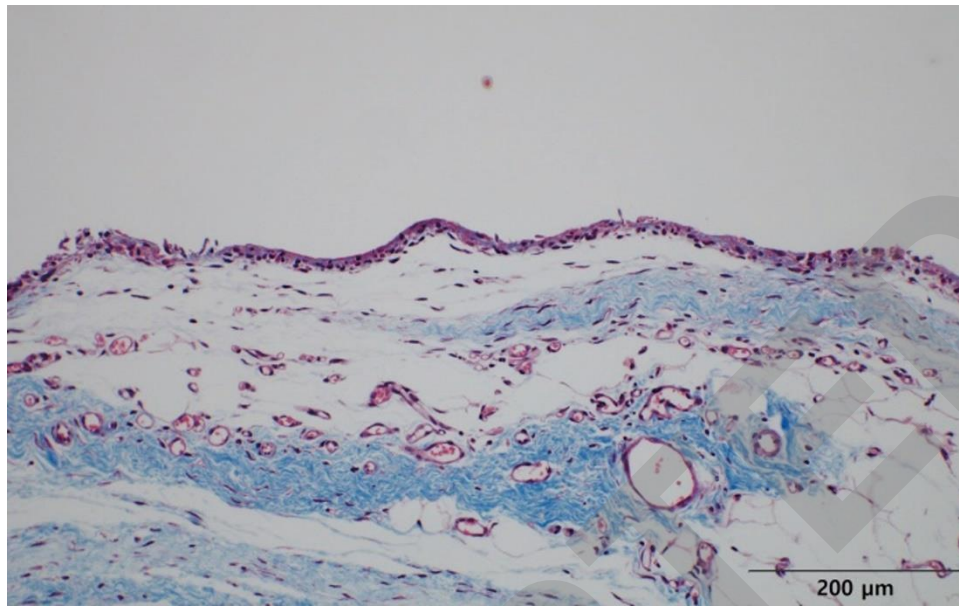


Figure 8C



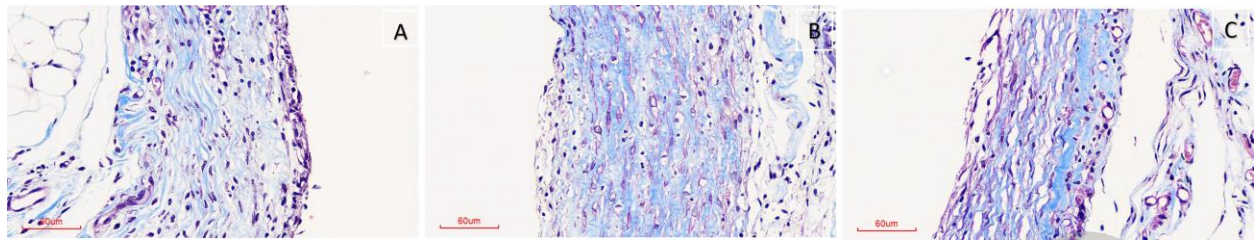
SDC Figure `1



Table. The sequence of the primers for qRT-PCR

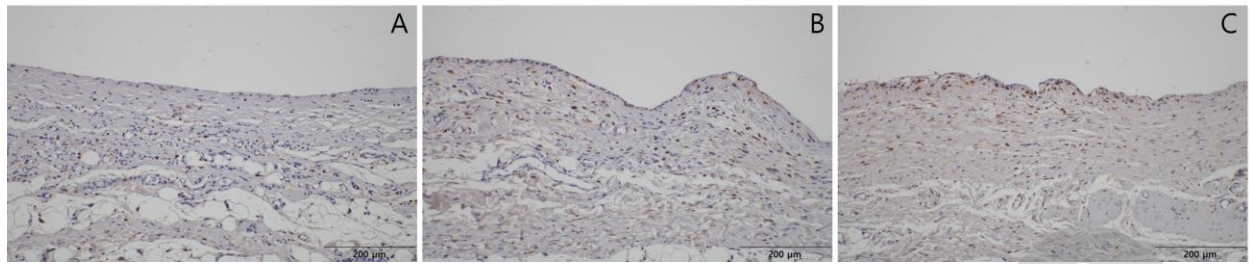
	Forward (5'-3')	Reverse (5'-3')
β -actin (housekeeping gene)	GATCTGGCACCACCTTCT	CTTTTCACGGTTGGCCTTAG
Collagen2A1	GGTGAGCCTGGTCAAACGG	ACTGTGTCCTTTCACGCCTTT
TNF- α	ATGAGCACTGAAAGCATGATCC	GAGGGCTGATTAGAGAGAGGTC
TGF β 2	TCGACATGGATCAGTTTATGCG	CCCTGGTACTGTTGTAGATGGA
IFN- γ	AAGACAACCAGGCCATCAGCAAC	GAACCTGGCGATGCTCATGAATGC
IL-4	CAAGGAACACCACGGAGAACGAG	CTTCAAGCACGGAGGTACATCACG
IL-6	AGGAGTGGCTAAGGACCAAGACC	TGCCGAGTAGACCTCATAGTGACC
IL-10	GTGAAGACCAGCAAAGGCCA	CTCTCGGAGCATGTGGGTCT
CD68	GGCGGTGGAATACAATGTGTCC	AGCAGGTCAAGGTGAACAGCTG
α -SMA	CTGACAGAGGCACCACTGAA	CATCTCCAGAGTCCAGCACA
Vimentin	CGGAAAGTGAATCCTTGCAGG	AGCAGTGAGGTCAGGCTTGGA
Collagen1A1	GGTGAGCCTGGTCAAACGG	ACTGTGTCCTTTCACGCCTTT

SDC Figure 3

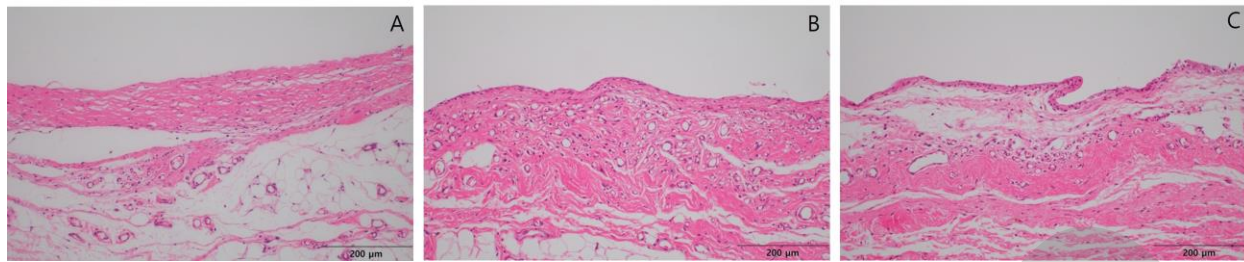


ACCEPTED

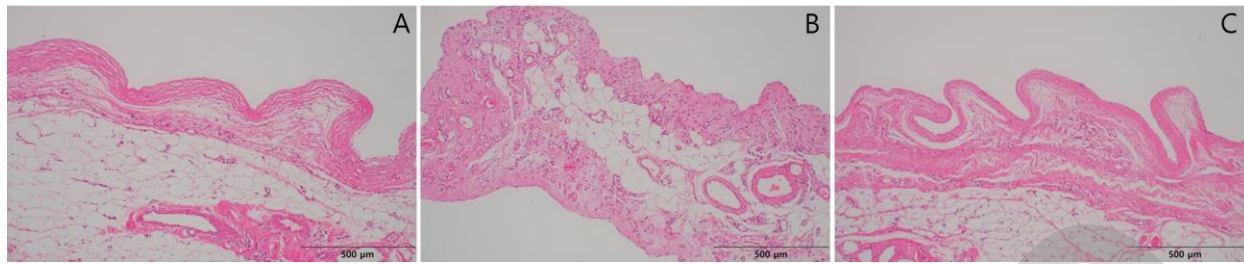
SDC Figure 4



SDC Figure 5



SDC Figure 6



ACCEPTED