# Evaluation of Multilayered Implantation with Collagen-Elastin Dermal Substitute (Matriderm®) in Nude Mouse

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# Evaluation of Multilayered Implantation with Collagen-Elastin Dermal Substitute (Matriderm®) in Nude Mouse

Directed by Professor Won Jai Lee

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Myung Chul Lee

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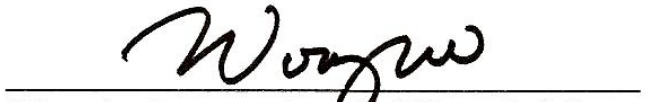
This certifies that the Master's Thesis of

Myung Chul Lee is approved.



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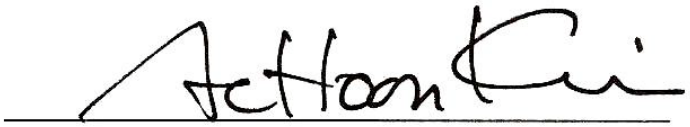

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Medical doctors including surgeons sometimes believe surgical procedures and operative treatments are superior to conducting experiments and looking after the data, however presentations and writings of competitors alert us to the importance of the research. As preparing for master's thesis, each processes and procedures, even mistakes and errors gave me lessons. We always look forward to fascinating results which have not been achieved by others, but such outcomes cannot be taken without helps and advices of mentors and senior colleagues. I appreciate to academic advisors, professor Won Jai Lee, Dong Kyun Rah and Se Hoon Kim whenever they correct my errors. Senior colleague, Dong Won Lee always taught me with passionate attitude as well as warm heartedness. I also feel thankful to researcher, Hye Jin Wang helping and staying next to me. Every single effort was fruitful and worthwhile academically.

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<**ABSTRACT**>

# Evaluation of Multilayered Implantation with Collagen-Elastin Dermal Substitute (Matriderm®) in Nude Mouse

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(Directed by Professor Won Jai Lee)

Materials for soft tissue augmentation as well as restoration of defective areas have been requested and developed continuously. Xenoplastic substitutes which can be applied without scarifying donor sites are noteworthy among various materials made up of autoplastic, alloplastic and synthetic substances. Matriderm® (Dr. Suwelack Skin & Health care AG, Billerbeck, Germany) is a matrix sheet consists of bovine type I collagen fiber template coated with α-elastin hydrolysate and can be utilized as a dermal substitute in case of full thickness skin defects or severe burn injuries. This study was

mapped out to investigate Matriderm® not only as a dermal substitute but also as a material for soft tissue augmentation. We studied the capability of bio-integration of the collagen-elastin dermal substitute (Matriderm®, Dr. Suwelack Skin & Health care AG, Billerbeck, Germany) when it was implanted being stacked into three layers, 6mm thickness. Matriderm® was also stacked into two layers for 4mm thickness as a control group and implanted subdermally on the dorsum of nude mice. Specimens of the inserted dermal substitute and the surrounding tissues were obtained from both the control group and the experiment group after certain periods: 3 days, 10 days, 20 days, 30 days and 40 days postoperatively. They underwent haematoxylin-eosin, CD 31 immunohistochemistry, Masson's trichrome and Verhoeff van Gieson staining. We observed alteration of thickness, degree of vasculogenesis through CD 31 immunohistochemistry staining, collagen and elastic fiber synthesis. 6mm thick Matriderm® had maintained its volume ratio as much as 4mm thick Matriderm® although thickness decreased throughout 6 weeks of experimental period. We expect they will be preserved constantly after being replaced by host derived collagen and elastic fibers. Vasculogenesis, collagen and elastic fiber synthesis took place fluently on the farthest portion from the host tissue at early stage of implantation. Collagen and elastic fibers became more concentrated as time passed and the architecture of Matriderm® has not been maintained anymore due to being replaced with newly formed collagen and elastic fibers in 40 days from implantation. The collagen fibers were randomly distributed as observed in healthy connective tissues, in constrast to pathologic appearance of scar tissues. In conclusion, Matriderm® is considered as an appropriate dermal matrix for tissue ingrowth and can be utilized as a new substitute for soft tissue augmentation. However, a research throughout sufficient period should precede a clinical application for the augmentation of soft tissues.

**Key words: Collagen-elastin dermal substitute, Thickness, Vasculogenesis, Collagen fiber, Elastic fiber**

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## I. INTRODUCTION

When there is a skin defect as a result of a burn, trauma, or surgical resection, it is highly difficult to expect a secondary healing if the defected area is large in size. Skin grafts are often implemented as a typical method to cover the defected area, however postoperative contracture due to insufficient dermal component of grafted skincan be troublesome. Recently, use of dermal matrix has become universal because it prevents secondary contracture or excavation in the grafted recipient site supplementing dermal component of defected areas.

Soft tissue restoration is not to be overlooked in reconstructive surgery as well as in cosmetic surgery. Soft tissue augmentation using autografts with such as fat, fascia, and dermis is considered most eligible because it does nottrigger immunological reactions, subsequently not causing inflammation. However it is flawed because of high morbidity rate and requirement of additional surgery. Therefore soft tissue restoration using alloplastic materials stands out in the respect that it helps overcome the previously mentioned disadvantages. Many different alloplastic materials have been introduced, among these; the method of soft tissue restoration using dermal matrix has gained the most popularity[1], because of minimizing the rejection compared to other types of alloplastic materials[2]. Also it is advantageous in that coalition with surrounding tissues is possible. In fact, in experiments using AlloDerm® during the wound healing, ingrowths of collagen fibers and angiogenesis has been observed[3]. Other dermal substitutes, such as lyophilized bovine pericardium (Lyoplant®) and irradiated bovine tendon (Hycure®) supported extensive vascular ingrowth and host fibroblast invasion[4]. Purified type I collagen from bovine Achilles tendon (DuraMatrix®) induced superficial fibroblastic infiltration and moderately thickened fibrous capsule with a mild to moderate inflammatory reaction[5].

Matriderm® (Dr. Suwelack Skin & Health care AG, Billerbeck, Germany) consists of bovine type I collagen fiber template coated with α-elastin hydrolysate derived from bovine ligamentum nuchae[2]. Matriderm® has been used for the treatment of skin defects adjacent to joint regions including the hand and wrist, the areas which need sufficient range of motion and revealed satisfactory results[6].

In this study, we observed alteration of thickness, vasculogenesis, collagen and elastic fiber synthesis when Matriderm® was implanted being stacked into two or three layers and investigated

whether Matriderm® can be utilized for soft tissue augmentation which have been used for grafts covering defected areas. In other words, according to the varying thicknesses of Matriderm®, this experiment studies whether tissue integration is possible in the areas far from the area in contact with the host tissue.

# II. MATERIALS AND METHODS

## 1. Laboratory Animal

This study implements nude mice (BALB/cAnNCrjBgi-nu/nu) authorized by Yonsei university medical center's institutional animal care and use committee. The laboratory animals are reared in the clean room at Yonsei university medical research center.

## 2. Experiment Group and Experimental Protocols

For 20 nude mice, their left sides make up the control group, and the right sides the experiment group. After anesthetizing the mouse by injecting Zolazepam® intraperitoneally, 15mm incisions were made on both sides 5mm parallel to the central line on the back. Adequate spaces were secured for implanting collagen elastin dermal substitute subdermally by dissecting 15 x 15$mm^2$ pockets. A 2mm-thick Matriderm® was cut into a square 10mm on each side horizontally and vertically. Two layers of Matriderm® were inserted on the left side and three on the right. After insertion, the incised skin was repaired with nylon #5-0 (Figure 1). After a time period after the insertion of two or three layered Matriderm®, four nude mice were performed euthanasia per the following schedule: 3 days, 10 days, 20 days, 30 days and 40 days. Then specimens of the inserted Matriderm® and the surrounding tissues were obtained from both the control group and the experiment group. With the specimens acquired from each time period, paraffin slices to include the skin, dermal substitute and subcutaneous tissue were made. Specific staining was carried out to

analyze each item: 1) hematoxylin-eosin stain for alteration of thickness, 2) CD31 immunohistochemistry stain for vasculogenesis, 3) Masson's trichrome stain for collagen fiber synthesis, and 4) Verhoeff van Gieson stain for elastic fiber synthesis.

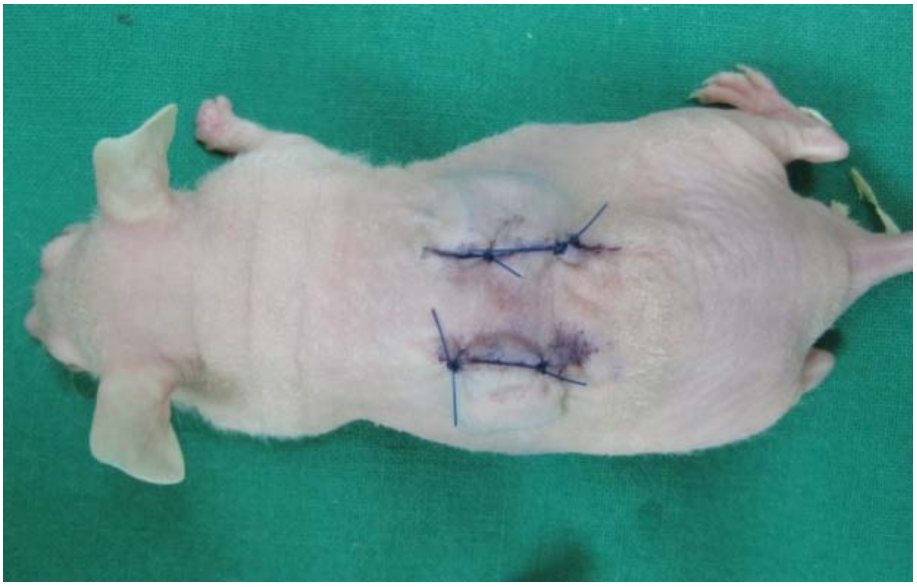


Fig 1. Implantation of Matriderm® on the dorsum of nude mouse. 2mm-thick 100mm$^2$-sized Matriderm® was implanted on the left side double layered (control group) and on the right side triple layered (experiment group) subdermally.

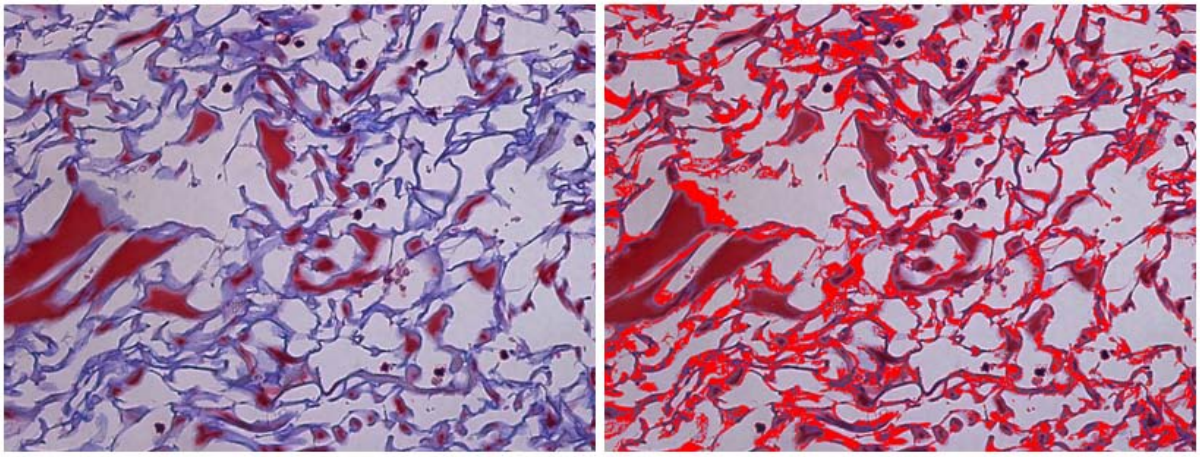
### 3. Methods of Staining and Measurement of Connective Tissue Density

Sections for analyzing vasculogenesis were incubated at 4°C overnight with anti-mouse platelet endothelial cell adhesion molecule-1 (PECAM/CD31) polyclonal antibody; M20, Santa Cruz Biotechnology, Santa Cruz, CA as primary antibodies and incubated at room temperature for 20 minutes with DAKO Envision Kit (DAKO) as secondary antibodies.

Staining solution for Masson's trichrome stain was prepared with bouin, weigert iron hematoxylin, 1% biebrich scarlet, phosphomolybdic-phosphotungstic acid and aniline solution.

Staining solution for Verhoeff van Gieson stain was prepared with alcoholic hematocylin, ferric chloride, weigert iodine, sodium thiosulfate, acid fuchsin and picric acid solution.

The density of synthesized collagen and elastic fibers was evaluated through semi-quantitative analysis using the MetaMorph® image analysis software (Universal Image Corp.) (Figure 2).



A. Collagen fibers B. MetaMorph® analysis

Fig 2. MetaMorph® image analysis. Collagen fibers were observed in blue color with Masson's trichrome stain (A). The blue color which represents collagen fibers were designated for analysis. The counterpart of collagen fibers were depicted as scarlet color with MetaMorph® image analysis program (B). The amount of pixels were calculated and recorded for quantification.

### 4. Alteration of Thickness of Matriderm® according to Time Periods

After hematoxylin-eosin staining, the thickness of the dermal substitutewas measured five times for each specimen and the average was recorded after each time period. The thickness was defined as vertical distance from the innermost to the outermost surface of Matriderm®. The trend of thickness was presented according to the time period for two layer group and three layer group each other. Furthermore, measured thickness was bisected for two layer group and trisected for three layer group, then results were presented and compared.

### 5. Vasculogenesis within Matriderm® according to Time Periods

In order to measure how much vasculogenesis occured within the collagen-elastin matrix, CD31 immunohistochemistry staining was performed. Vascular tissues, being observed through a 400x optical microscope, which were covered with a single-layered endothelial cell, and did not have a muscular layer were interpreted as capillaries. For each slide, the number of blood vessels was measured five times in the central line area farthest away from the surface of the dermal substitute, then the average of vascular density (the number of vessels/OPF) was written down. The trend of vascular density was presented according to the time period for two layer and three layer group each other.

### 6. Collagen and Elastic Fiber Synthesis within Matriderm® according to Time Periods

In order to measure elastic fiber and collagen fiber for the specimen acquired from each time period,

Masson's trichrome and Verhoeff van Gieson (VVG) staining were performed. The arrangement and the degree of presence of collagen fibers and elastic fibers were observed in the central-line area farthest away from the surface of implanted dermal substitute. A quantitative analysis of the density of collagen fibers and elastic fibers was executed through MetaMorph® analysis program. The density was measured five times in the central line area farthest away from the surface of the dermal substitute, then the average of density (pixels/OPF) was recorded. The trend of the density was presented according to the time period for two layer and three layer group each other. Matriderm® sheets which have not been implanted were also stained for observing the arrangement and density of collagen and elastic fibers within the matrix alone.

### 7. Statistical Analysis

Paired T-test was used for comparing the thickness, the number of new blood vessels, the quantity of collagen and elastic fibers between two layer and three layer groups. One-way ANOVA (Analysis of Variance) was also used to test if there is any difference between time periods on those variables at each layer group. The significant ANOVA results were followed by the post-hoc test for pairwise comparisons adjusted by Bonferroni correction. The statistical significance was evaluated with a 95% confidence interval.

# III. RESULTS

## 1. Thickness

The architecture of Matriderm® maintained in 10 days after implantation, however the structure was not preserved anymore as time passed until 40th day. The original matrix which consists of collagen and elastin is substituted with collagen and elastic fibers produced by host derived fibroblasts (Fig 3, 4). The average thickness of the two layer group was 2.55 ± 0.12 mm after the 3rd day of implantation and 1.76 ± 0.14 mm after 40th day of implantation.The average thickness of the three layer group was 3.86 ± 0.11 mm after the 3rd day of implantation and 2.45 ± 0.14mm after 40th day of implantation (Table 1). The thickness of the three layer group maintained superiorly to the two layer group significantly ($p< 0.05$). The thickness decreased as time passed on both three layer and two layer group, furthermore difference between 20th day and 30th day was prominent on both group significantly ($p< 0.05$, two layer group; $p< 0.01$, three layer group) (Fig 5). We compared thickness of single layer of each group. The average thickness of single layer for the two layer group was 1.27 ± 0.06mm after the 3rd day of implantation and 0.88 ± 0.07 mm after 40th day of implantation. The average thickness of single layer for the three layer group was 1.28 ± 0.03 mm after the 3rd day of implantation and 0.81 ± 0.04 mm after 40th day of implantation (Table 2). The thickness of single layer did not reveal significant difference between three layer and two layer group during the experimental period ($p> 0.05$), which means the middle layer among three layers was preserved as much as the each layer of the two layer group (Fig 3, 4, 6). The thickness of single layer decreased as

time passed on both groups revealing significant difference between $20^{th}$ and $30^{th}$ day ($p< 0.05$, two layer group; $p< 0.01$, three layer group).

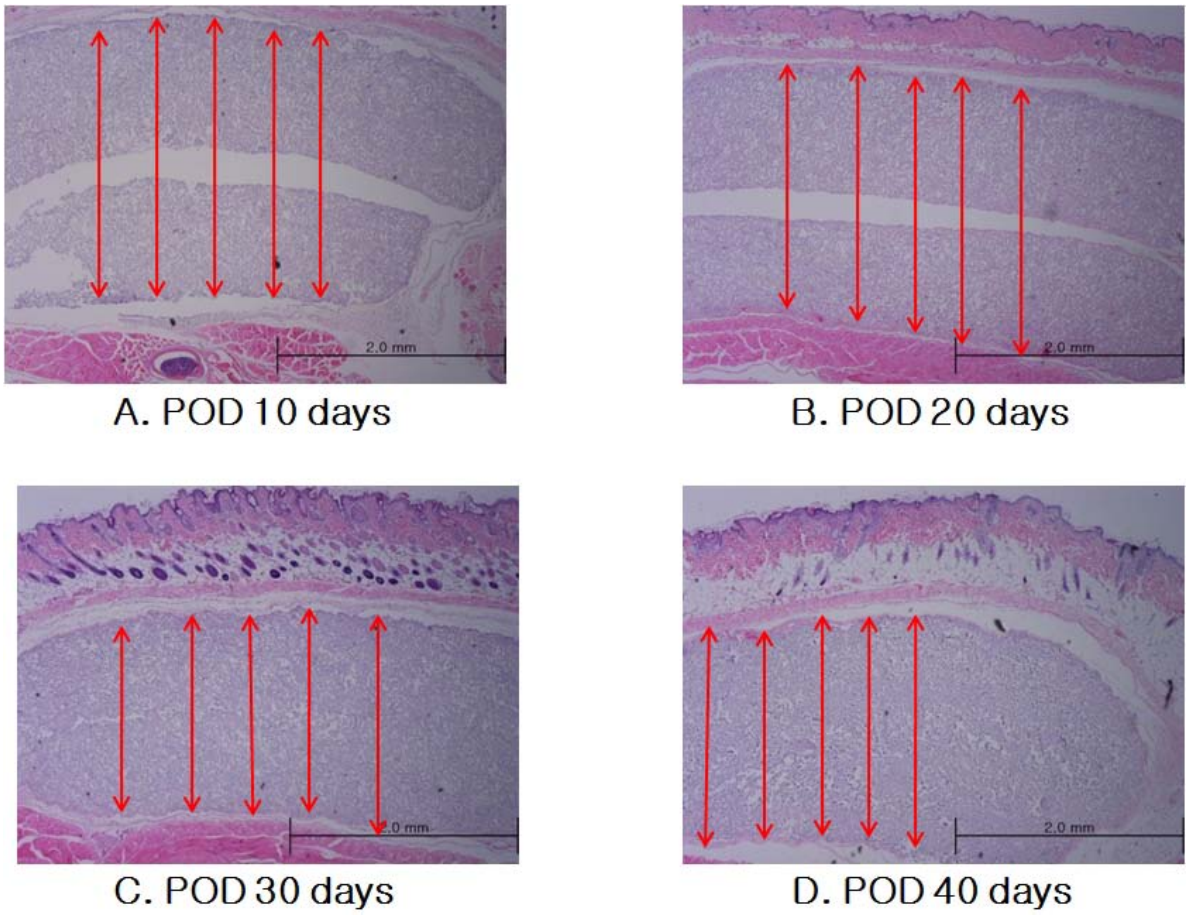



Fig 3. Measurement of thickness for two layer group (HE stain, x40). The thickness of the dermal substitute was measured five times for each specimen and the average was recorded after each time period. The architecture of Matriderm® maintained in 10 days after implantation (A), however the structure was not preserved anymore as time passed until 40th day (D). The original matrix which consists of collagen and elastin is substituted with collagen and elastic fibers produced by host derived fibroblasts.

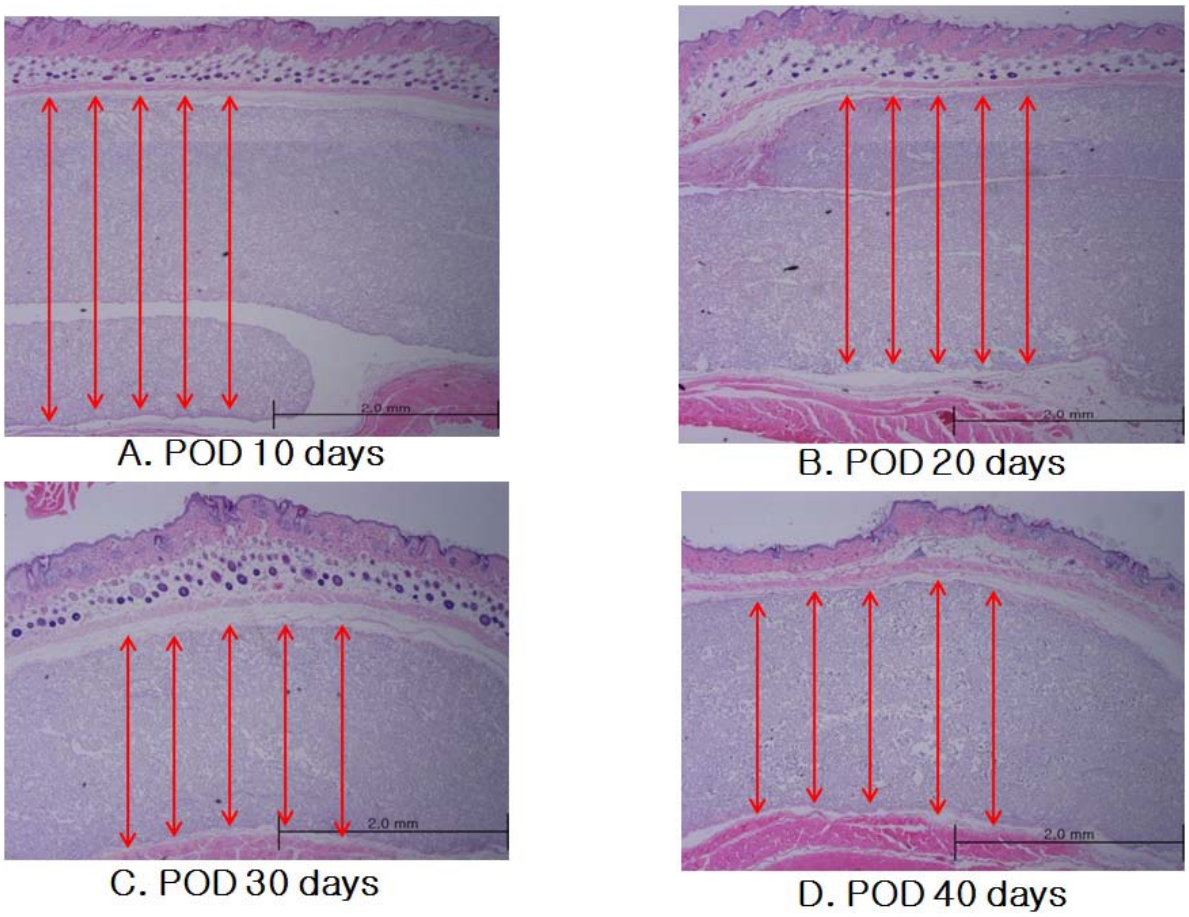



Fig 4. Measurement of thickness for three layer group (HE stain, x40). The architecture of Matriderm® maintained in 10 days after implantation, however the structure was not preserved anymore as time passed until 40th day as observed in two layer group.

Table 1. Thickness according to Time Period (mm ± SD)

| | 3 days | 10 days | 20 days | 30 days | 40 days |
|---|---|---|---|---|---|
| 2 layer group | 2.55 ± 0.12 | 2.46 ± 0.14 | 2.41 ± 0.21 | 1.92 ± 0.36 | 1.76 ± 0.15 |
| 3 layer group | 3.86 ± 0.11 | 3.73 ± 0.16 | 3.56 ± 0.25 | 2.66 ± 0.11 | 2.45 ± 0.14 |
| *P* value | < 0.005 | < 0.001 | < 0.05 | < 0.01 | < 0.001 |

The thickness of the three layer group maintained superiorly to the two layer group significantly ($p<$ 0.05). The thickness decreased as time passed on both three layer and two layer group, furthermore difference between $20^{th}$ day and $30^{th}$ day was prominent on both group significantly ($p<$ 0.05, two layer group; $p<$ 0.01, three layer group).

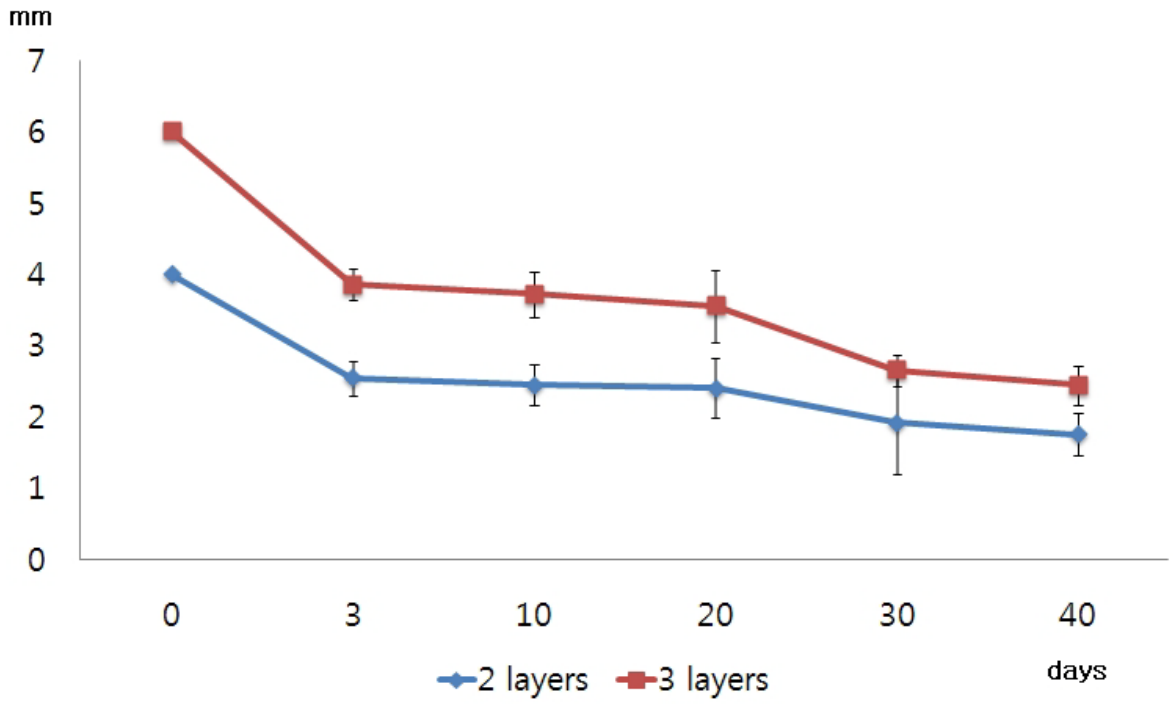



Fig 5. The thickness according to time period and comparison of thickness between two layer and three layer group.The thickness of the three layer group maintained superiorly to the two layer group significantly ($p< 0.05$). The thickness decreased as time passed on both three layer and two layer group, furthermore difference between $20^{th}$ day and $30^{th}$ day was prominent on bothgroup significantly ($p< 0.05$, two layer group; $p< 0.01$, three layer group).

Table 2. The Thickness of Single Layer according to Time Period (mm ± SD)

| | 3 days | 10 days | 20 days | 30 days | 40 days |
|---|---|---|---|---|---|
| 2 layer group | 1.27 ± 0.06 | 1.23 ± 0.07 | 1.20 ± 0.09 | 0.95 ± 0.18 | 0.88 ± 0.07 |
| 3 layer group | 1.28 ± 0.03 | 1.24 ± 0.05 | 1.18 ± 0.08 | 0.88 ± 0.03 | 0.81 ± 0.04 |
| *P* value | > 0.05 | > 0.05 | > 0.05 | > 0.05 | > 0.05 |

Measured thickness was bisected for two layer group and trisected for three layer group. The thickness of single layer did not reveal significant difference between three layer and two layer group during the experimental period ($p > 0.05$), which means the middle layer among three layers was preserved as much as the each layer of the two layer group. The thickness of single layer decreased as time passed on both groups revealing significant difference between $20^{th}$ and $30^{th}$ day ($p < 0.05$, two layer group; $p < 0.01$, three layer group).

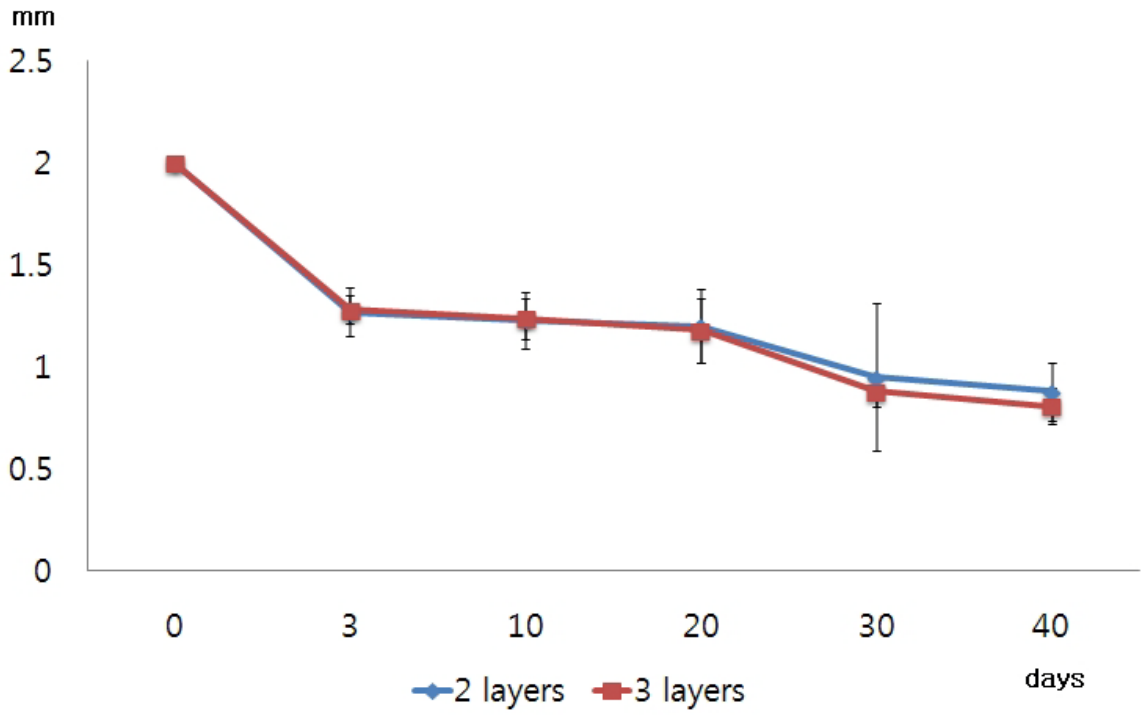



Fig 6. The thickness of single layer according to time period and comparison of thickness between two layer and three layer group. The thickness of single layer did not reveal significant difference between three layer and two layer group during the experimental period ($p > 0.05$), which means the middle layer among three layers was preserved as much as the each layer of the two layer group. The thickness of single layer decreased as time passed on both groups revealing significant difference between 20$^{th}$ and 30$^{th}$ day ($p < 0.05$, two layer group; $p < 0.01$, three layer group).

## 2. Vasculogenesis

The area of CD 31 positive implicates with vasculogenesis. The average number of blood vessels for the two layer group was 1.07 ± 0.33 /OPF after the 3$^{rd}$ day of implantation and 8.97 ± 1.26 /OPF after 40$^{th}$ day of implantation. The average number of blood vessels for the three layer group was 1.45 ± 0.21 /OPF after the 3$^{rd}$ day of implantation and 9.3 ± 1.09 /OPF after 40$^{th}$ day of implantation (Table 3). The number of blood vessels increased as time passed, furthermore the number revealed significant difference between 20$^{th}$ and 30$^{th}$ day ($p< 0.01$, two layer group; $p< 0.01$, three layer group); and between 30$^{th}$ and 40$^{th}$ day ($p< 0.05$, two layer group; $p< 0.01$, three layer group). There was no significant difference between two layer and three layer group ($p> 0.05$), meaning adequate vasculogenesis of middle layer among three layers as each layers of two layer group (Fig7, 8, 9).

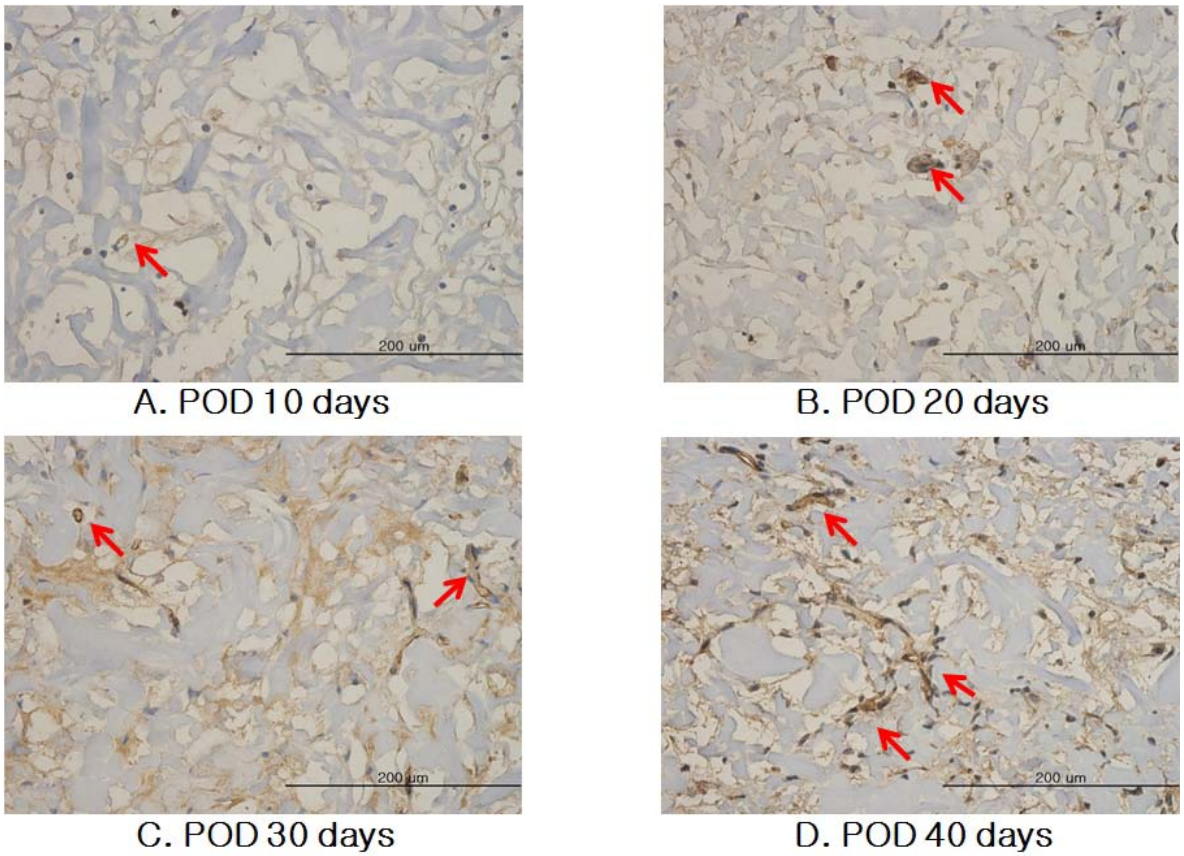



Fig 7. Measurement of vasculogenesis for two layer group (CD31 immunohistochemistry stain, x400). Vascular tissues which were covered with a single-layered endothelial cell, and did not have a muscular layer were interpreted as capillaries (arrow). The number of blood vessels was measured five times in the central line area farthest away from the surface of the dermal substitute, then the average of vascular density (the number of vessels/OPF) was recorded. The number of blood vessels increased as time passed.

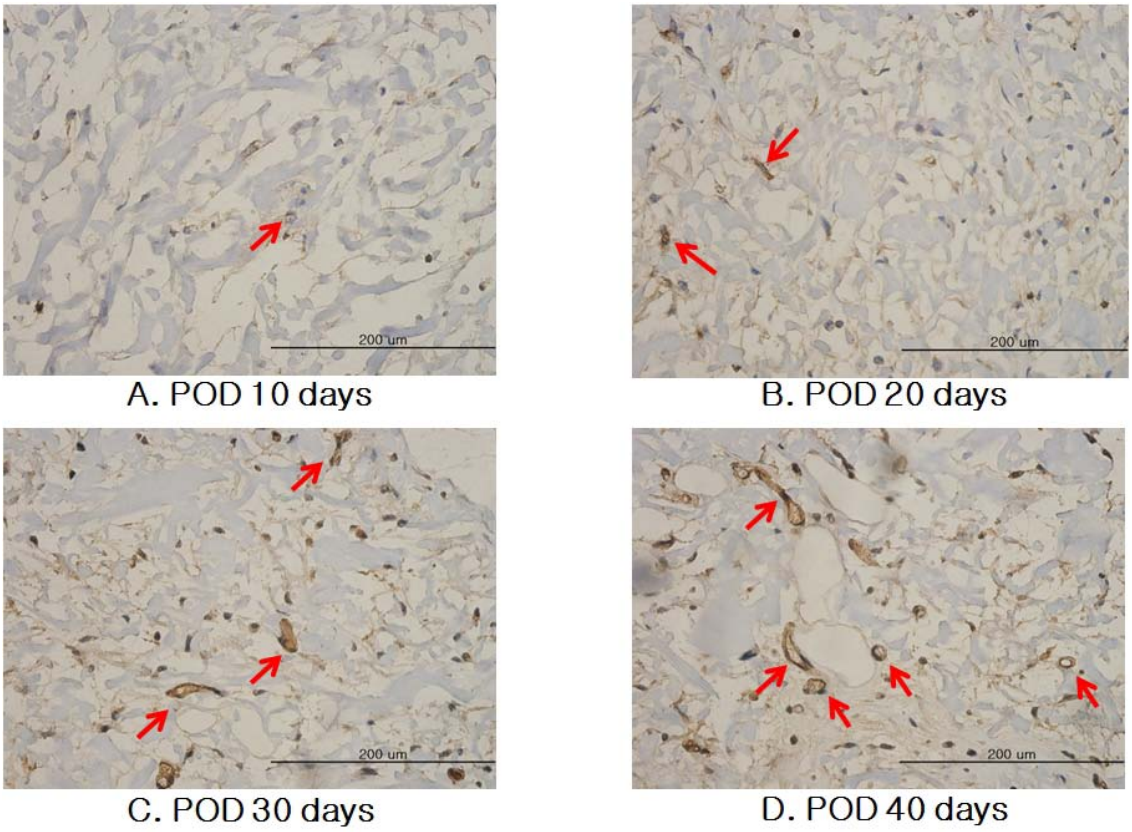



Fig 8. Measurement of vasculogenesis for three layer group (CD31 immunohistochemistry stain, x400). The number of blood vessels increased as time passed as observed in two layer group.

Table 3. The Number of Blood Vessels according to Time Period (number of vessels/OPF ± SD)

| | 3 days | 10 days | 20 days | 30 days | 40 days |
|---|---|---|---|---|---|
| 2 layer group | 1.07 ± 0.33 | 3.19 ± 0.42 | 4.41 ± 1.04 | 6.67 ± 0.87 | 8.97 ± 1.26 |
| 3 layer group | 1.45 ± 0.21 | 4.02 ± 0.29 | 4.27 ± 0.61 | 6.32 ± 0.91 | 9.31 ± 1.09 |
| *P* value | > 0.05 | > 0.05 | > 0.05 | > 0.05 | > 0.05 |

There was no significant difference between two layer and three layer group ($p > 0.05$), meaning adequate vasculogenesis of middle layer among three layers as each layers of two layer group. The number of blood vessels increased as time passed, furthermore the number revealed significant difference between $20^{th}$ and $30^{th}$ day ($p < 0.01$, two layer group; $p < 0.01$, three layer group); and between $30^{th}$ and $40^{th}$ day ($p < 0.05$, two layer group; $p < 0.01$, three layer group).

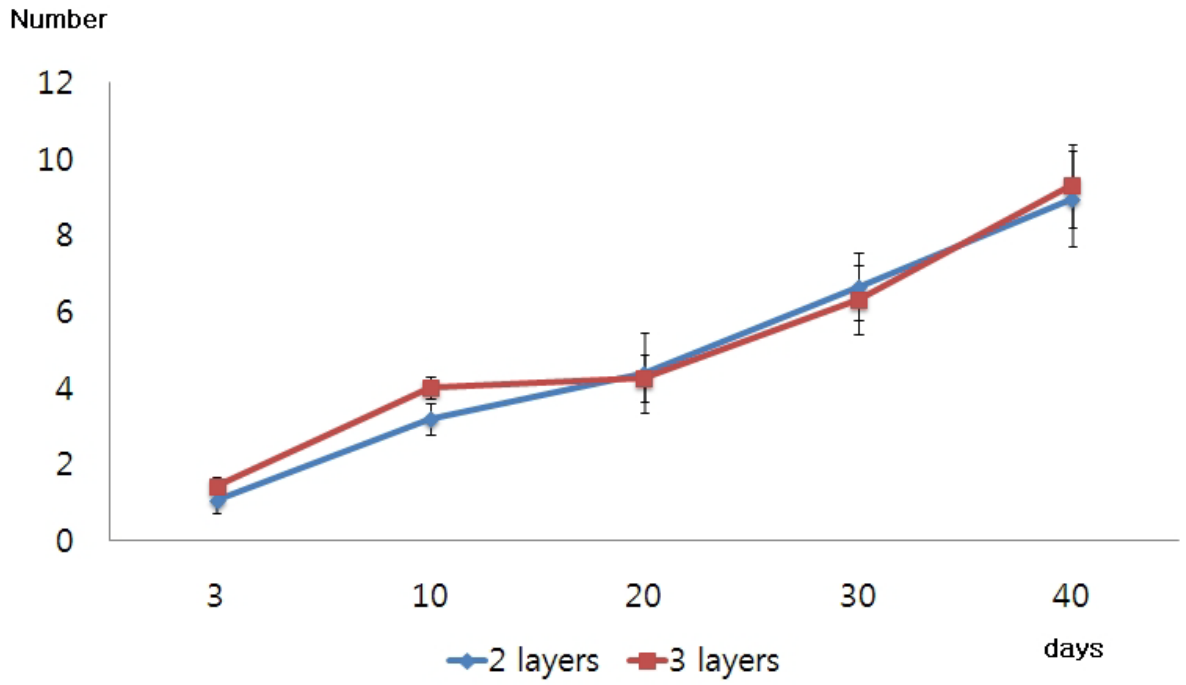



Fig 9. The number of blood vessels according to time period and comparison of vascular density between two layer and three layer group. The number of blood vessels increased as time passed, furthermore the number revealed significant difference between 20$^{th}$ and 30$^{th}$ day ($p$< 0.01, two layer group; $p$< 0.01, three layer group); and between 30$^{th}$ and 40$^{th}$ day ($p$< 0.05, two layer group; $p$< 0.01, three layer group). There was no significant difference between two layer and three layer group ($p$> 0.05), meaning adequate vasculogenesis of middle layer among three layers as each layers of two layer group.

### 3. Collagen Fiber

The Collagen fibers became more concentrated as time passed and the architecture of Matriderm® did not maintain anymore due to being replaced with newly formed collagen fibers in 40 days. Few collagen fibers existed within the matrix before being synthesized by host derived fibroblasts. The arrangement of collagen fibers was irregular as observed in healthy connective tissues, which is one of the advantages of Matriderm® (Fig 10, 11).

Quantitative measurement of collagen fibers was done using MetaMorph® analysis program. The average density of collagen fibers for the two layer group was 37362 ± 3149 pixels/OPF after the 3$^{rd}$ day of implantation and 60729 ± 2423 pixels/OPF after 40$^{th}$ day of implantation. The average density of collagen fibers for the three layer group was 24354 ± 2461 pixels/OPF after the 3$^{rd}$ day of implantation and 60103 ± 3714 pixels/OPF after 40$^{th}$ day of implantation (Table 4). The amount of collagen fibers increased as time passed, furthermore the amount revealed significant difference between 3$^{rd}$ and 10$^{th}$ day ($p< 0.05$, two layer group; $p< 0.05$, three layer group); between 20$^{th}$ and 30$^{th}$ day for three layer group ($p< 0.05$); and between 30$^{th}$ and 40$^{th}$ day for two layer group ($p< 0.05$). There was no significant difference between two layer and three layer group ($p> 0.05$) meaning adequate collagen synthesis of middle layer among three layers (Fig 10, 11, 12).

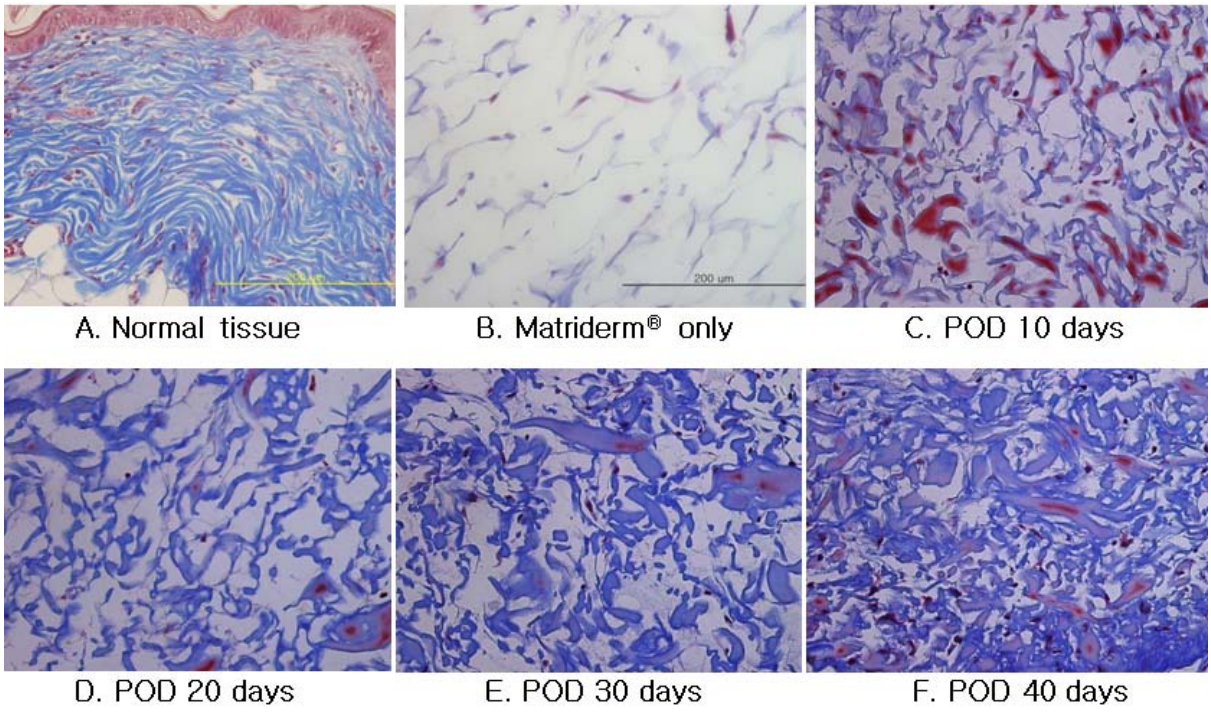



Fig 10. Evaluation of collagen fibers for two layer group (Masson's trichrome stain, x400). The arrangement and the degree of presence of collagen fibers were observed in the central-line area farthest away from the surface of implanted dermal substitute and normal subcutaneous tissue of a nude mouse. Moderate amount of collagen fibers have existed in the subcutaneous tissue (A). Few collagen fibers were recognized within the matrix before being synthesized by host derived fibroblasts (B). The collagen fibers became more concentrated as time passed and the architecture of Matriderm® did not maintain anymore due to being replaced with host derived collagen fibers in 40 days (F). The arrangement of collagen fibers was irregular as observed in healthy connective tissues, which is one of the advantages of Matriderm®.

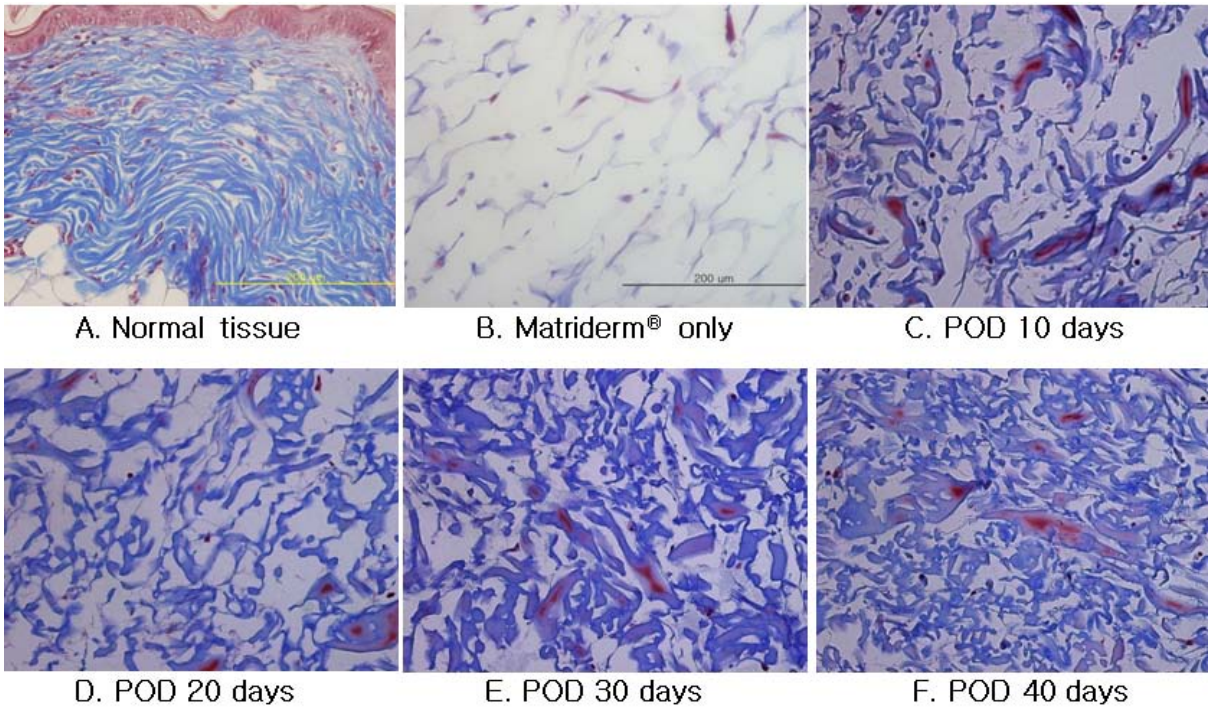



Fig 11. Evaluation of collagen fibers for three layer group (Masson's trichrome stain, x400). Moderate amount of collagen fibers have existed in the subcutaneous tissue (A). Few collagen fibers were recognized within the matrix before being synthesized by host derived fibroblasts (B). Collagen fibers became more concentrated as time passed and the arrangement of collagen fibers was irregular as observed in two layer group.

Table 4. Density of Collagen Fibers according to Time Period (pixels/OPF ± SD)

| | 3 days | 10 days | 20 days | 30 days | 40 days |
|---|---|---|---|---|---|
| 2 layer group | 37362 ± 3149 | 48727 ± 4675 | 49825 ± 1592 | 53148 ± 3661 | 60729 ± 2423 |
| 3 layer group | 24354 ± 2461 | 43792 ± 3167 | 44568 ± 4268 | 54189 ± 5109 | 60103 ± 3714 |
| *P* value | > 0.05 | > 0.05 | > 0.05 | > 0.05 | > 0.05 |

Quantitative measurement of collagen fibers was done using MetaMorph® analysis program. There was no significant difference between two layer and three layer group ($p > 0.05$) meaning adequate collagen synthesis of middle layer among three layers. The amount of collagen fibers increased as time passed, furthermore the amount revealed significant difference between 3rd and 10th day ($p < 0.05$, two layer group; $p < 0.05$, three layer group); between 20th and 30th day for three layer group ($p < 0.05$); and between 30th and 40th day for two layer group ($p < 0.05$).

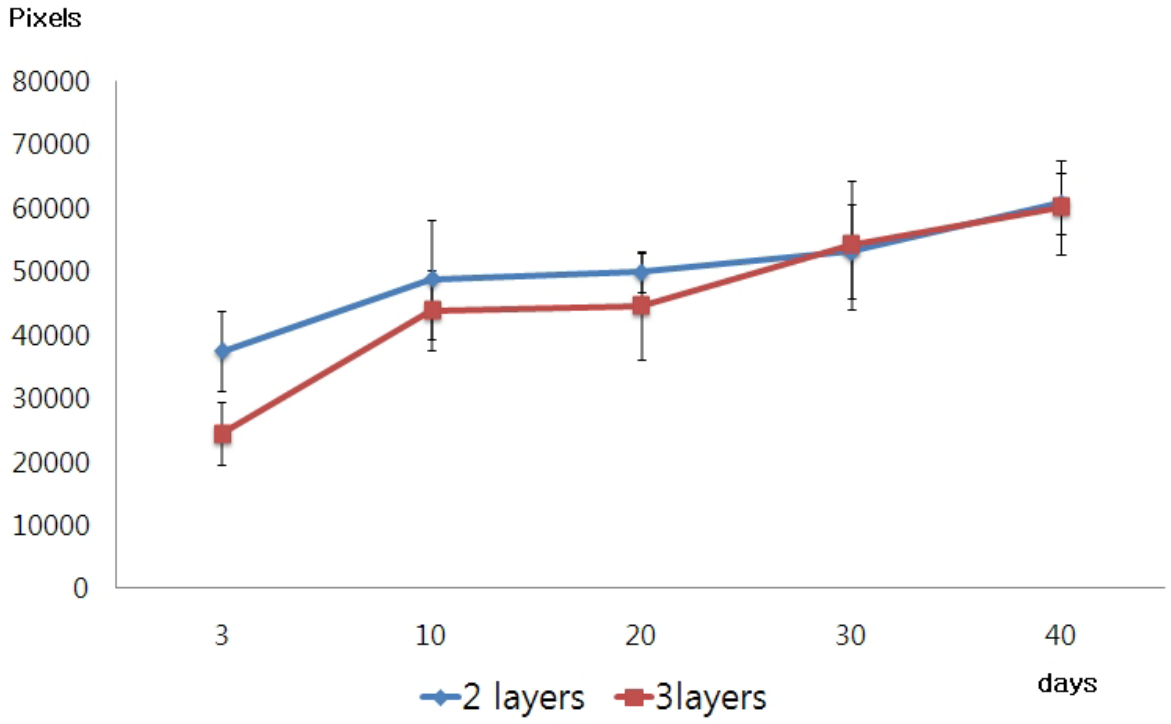



Fig 12. The density of collagen fibers according to time period and comparison of vascular density between two layer and three layer group. The amount of collagen fibers increased as time passed, furthermore the amount revealed significant difference between 3$^{rd}$ and 10$^{th}$ day ($p$< 0.05, two layer group; $p$< 0.05, three layer group); between 20$^{th}$ and 30$^{th}$ day for three layer group ($p$< 0.05); and between 30$^{th}$ and 40$^{th}$ day for two layer group ($p$< 0.05). There was no significant difference between two layer and three layer group ($p$> 0.05) meaning adequate collagen synthesis of middle layer among three layers.

## 4. Elastic Fiber

Elastic fibers were observed more and more with Verhoeff van Gieson staining as time passed. Few elastic fibers existed within the matrix before being synthesized by host derived fibroblasts (Fig 13, 14). Matriderm® consists of collagen matrix and elastin as distinguished from other dermal matrix and substitutes. The elastin existing within Matriderm® contributes to elastic fiber synthesis.

Quantitative measurement of elastic fibers was done using MetaMorph® analysis program. The average density of elastic fibers for the two layer group was 34670 ± 3635 pixels/OPF after the 3$^{rd}$ day of implantation and 62793 ± 4127 pixels/OPF after 40$^{th}$ day of implantation. The average density of elastic fibers for the three layer group was 34948 ± 3172 pixels/OPF after the 3$^{rd}$ day of implantation and 69237 ± 3719 pixels/OPF after 40$^{th}$ day of implantation (Table 5). The amount of elastic fibers increased as time passed, furthermore the amount revealed significant difference between 3$^{rd}$ and 10$^{th}$ day for two layer group ($p< 0.05$); between 10$^{th}$ and 20$^{th}$ day for three layer group ($p< 0.05$); and between 30$^{th}$ and 40$^{th}$ day ($p< 0.05$, two layer group; $p< 0.05$, three layer group). There was no significant difference between two layer and three layer group ($p> 0.05$), which means adequate elastic fiber synthesis of middle layer among three layers (Fig 13, 14, 15).

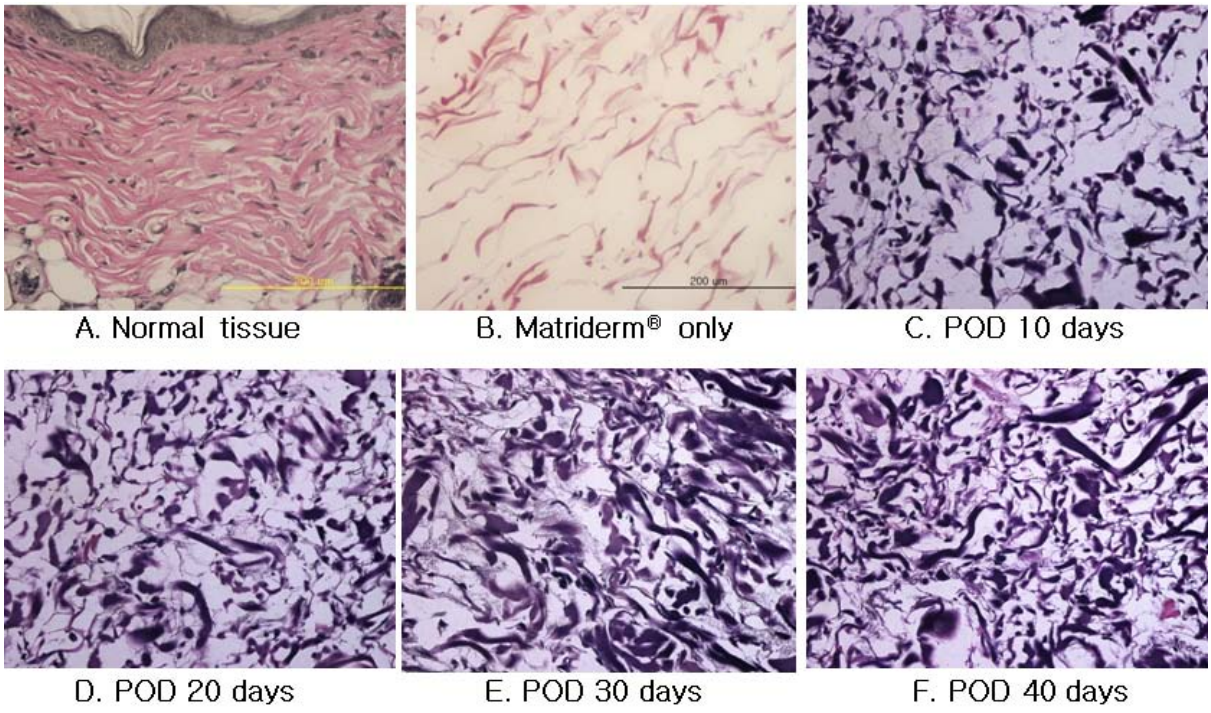



Fig 13. Evaluation of elastic fibers for two layer group (Verhoeff van Gieson stain, x400). The arrangement and the degree of presence of elastic fibers were observed in the central-line area farthest away from the surface of implanted Matriderm®. Moderate amount of elastic fibers have existed in the normal subcutaneous tissue of a nude mouse (A). Few elastic fibers were recognized within the matrix before being synthesized by host derived fibroblasts (B). Elastic fibers were observed more and more as time passed. Matriderm® consists of collagen matrix and elastin as distinguished from other dermal matrix and substitutes. The elastin existing within Matriderm® contributes to elastic fiber synthesis.

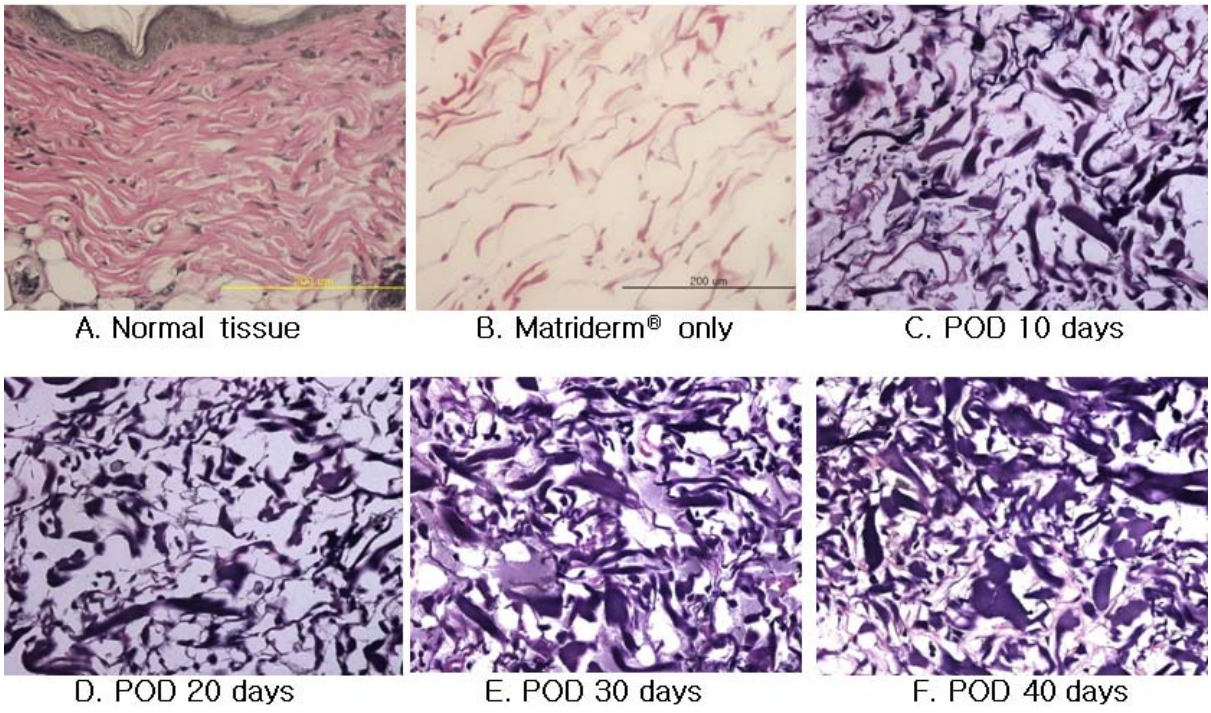



Fig 14. Evaluation of elastic fibers for three layer group (Verhoeff van Gieson stain, x400). Moderate amount of elastic fibers have existed in the subcutaneous tissue (A). Few elastic fibers were recognized within the matrix before being synthesized by host derived fibroblasts (B). Elastic fibers were arranged dispersed and observed more and more as time passed.

Table 5. Density of Elastic Fibers according to Time Period (pixels/OPF ± SD)

| | 3 days | 10 days | 20 days | 30 days | 40 days |
|---|---|---|---|---|---|
| 2 layer group | 34670 ± 3635 | 45692 ± 2481 | 54479 ± 5274 | 56439 ± 2896 | 62793 ± 4127 |
| 3 layer group | 34948 ± 3172 | 37629 ± 4326 | 51044 ± 4749 | 55273 ± 3426 | 69237 ± 3719 |
| *P* value | > 0.05 | > 0.05 | > 0.05 | > 0.05 | > 0.05 |

Quantitative measurement of elastic fibers was done using MetaMorph® analysis program. There was no significant difference between two layer and three layer group ($p > 0.05$), which means adequate elastic fiber synthesis of middle layer among three layers. The amount of elastic fibers increased as time passed, furthermore the amount revealed significant difference between 3rd and 10th day for two layer group ($p < 0.05$); between 10th and 20th day for three layer group ($p < 0.05$); and between 30th and 40th day ($p < 0.05$, two layer group; $p < 0.05$, three layer group).

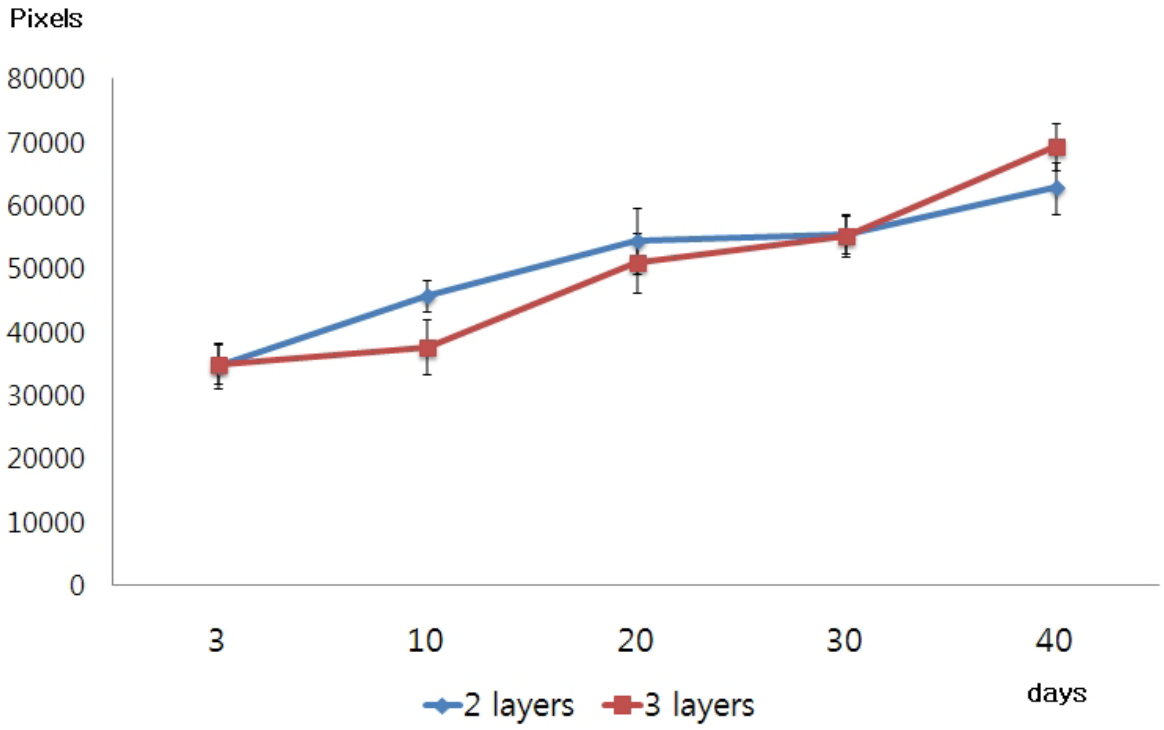



Fig 15. The density of elastic fibers according to time period and comparison of vascular density between two layer and three layer group. The amount of elastic fibers increased as time passed, furthermore the amount revealed significant difference between 3$^{rd}$ and 10$^{th}$ day for two layer group ($p$<0.05); between 10$^{th}$ and 20$^{th}$ day for three layer group ($p$<0.05); and between 30$^{th}$ and 40$^{th}$ day ($p$<0.05, two layer group; $p$<0.05, three layer group). There was no significant difference between two layer and three layer group ($p$>0.05), which means adequate elastic fiber synthesis of middle layer among three layers.

# IV. DISCUSSION

Materials available for soft tissue augmentation of contour defects have been introduced throughout decades. The ideal material should match the surrounding tissue in texture, pliability and color; neither transmit nor cause any disease in the recipient; and persist and ultimately be integrated into the host tissues. As tissue engineering advanced, materials for restoring large volume defect have been studied. The purpose of this study was to compare the volumetric and histologic changes of the collagen elastin dermal substitute, Matriderm® implanted subcutaneously in nude mice. The collagen elastin dermal substitute, Matriderm® (Dr. Suwelack Skin and Health Care AG, Billerbeck, Germany) is a highly porous, 2-mm-thick membrane consisting of a native bovine type I collagen fiber template coated with α-elastin hydrolysate derived from bovine ligamentum nuchae in a concentration of 3 weight-to-weight percent ratio. The matrices were treated with γ-irradiation (1000 Gy) and stored at room temperature[2]. Matriderm® has been used for the treatment of skin defects adjacent to joint regions including the hand and wrist, and the areas which need sufficient range of motion[7]. Hasliket al[8] reported superior elasticity and satisfactory outcome after surgical procedures using Matriderm® in the hand and wrist region. We expected elastic and pliable characteristics of Matriderm® resistant to contracture would make for adequate survival when it was implanted for augmentation.

The thickness decreased to 62.5% on the 3rd day, 42.5% on the 40th day in three layer group and 65% on the 3rd day, 40% on the 40th day in two layer group. Previous studies revealed 57.2% of survival for AlloDerm®, 83.8% for autologous cartilage and 68.6% for bovine pericardium on the date after 8th week postoperatively[4]. The rate of thickness maintenance was lower than other dermal substitute

materials, it is due to coarse and loose structure, softness after hydration manipulating for implantation and pressure of adjacent tissue after implantation. Comparison of thickness in single layer of each groups did not show significant difference as time passed meaning the middle layer among three layers was preserved as much as the each of two layers. We anticipate thickness will maintain constantly after host derived collagen, elastic fibers replace the whole collagen-elastic matix of Matriderm®. Experiments observing the results for sufficient period should precede the clinical application.

Vasculogenesis has progressed significantly within the collagen-elastin matrix on CD 31 immunohistochemistry stain. CD 31, as known as platelet endothelial cell adhesion molecule, is expressed on the surface of endothelial cell, platelet and monocyte. Endothelial ingrowth was observed during serum imbibitions period and revascularization was identified 9 days after skin graft. Reasonable vasculogenesis in early stage proved superior property of Matriderm® as an adequate matrix for migration of host derived angiogenetic cells.

Collagen fibers produced by fibroblasts constitute extracellular matrix and function as the scaffold. Progressive increase of collagen fibers was observed, furthermore dense collagen stroma was identified eventually in both two layer and three layer groups. Collagen fibers were distributed loosely and randomly, as opposed to pathologic findings of a tissue with scar contracture which has parallel and regular distribution.

Elastic fibers are synthesized with glycoprotein, fibrillin, elastin by fibroblasts and smooth muscle cells. Elastic fibers were formed sufficiently in both two layer and three layer groups, which proved Matriderm® to be adequate matrix for connective tissue ingrowth. Resilient characteristic of elastic

fibers can decrease wound contracture along with randomized loose distribution of collagen fibers[9].

Waaijman et al[10] seeded large numbers of proliferating epidermal cells (keratinocytes and melanocytes) onto a three dimensional matrix composed of elastin and collagen types I, III, and V (Matriderm®), which enabled easy and stable transport of the epidermal cells under ambient conditions. Keck et al[11] conducted research to determine whether keratinocytes and preadipocytes grow simultaneously on a bovine derived collagen-elastin matrix (Matriderm®) under in vitro conditions in order to obtain a multi layer skin substitute. Human keratinocytes as well as human preadipocytes were seeded onto a collagen-elastin matrix (Matriderm®). Simultaneous growth of keratinocytes and preadipocytes was observed on the collagen-elastin matrix. Keratinocytes adhered well to the surface of the matrix and formed a confluent epidermis like layer. Preadipocytes adhered well and also penetrated into the deeper layers of the matrix, which represents that Matriderm® is suitable matrix for migration of progenitor cells and formation of mature cells. Further experiments using Matriderm® with variable thickness and density should be conducted for obtaining more advanced outcomes, furthermore inventing more practical materials for soft tissue augmentation.

# V. CONCLUSION

We investigated the survival and reviewed histological analysis of 6mm thick collagen-elastin dermal substitute (Matriderm®) when it was implanted subdermally on the dorsum of nude mouse.

1. Six-milimeter thick Matriderm® had maintained its volume ratio as much as 4mm thick Matriderm® although thickness decreased throughout 6 weeks of experimental period. We expect they will be preserved constantly after being replaced by host derived collagen and elastic fibers.
2. Vasculogenesis, collagen and elastic fiber synthesis took place fluently on the farthest portion from the host tissue at early stage of implantation.
3. A research throughout sufficient period should precede a clinical application for the augmentation of soft tissues.

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**ABSTRACT (IN KOREAN)**

# 누드 마우스에서 다층 이식한 진피 대체물 (Matriderm®)이 생착되는 정도의 평가

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이명철

연조직 보강과 결손부위의 복원을 위한 대체 물질은 지속적으로 연구되고 개발되어왔다. 공여부가 손상되지 않은 채 결손된 수혜부를 복원할 수 있는 이형물질이 각광받고 있다. 실험군으로 2mm 두께의 콜라겐-엘라스틴 진피 대체물인 Matriderm® (Dr. Suwelack Skin & Health care AG, Billerbeck, Germany) 을 3겹으로 쌓아 총 6mm 두께 Matriderm® 을 누드 마우스 배부의 중심선 우측에 이식하여, 생착되는 정도를 연구하였다. 대조군으로 Matriderm® 을 2겹으로 쌓아 총 4mm 두께 Matriderm® 을 중심선

좌측에 이식하였다. 이식 후 3일, 10일, 20일, 30일, 40일 경과하여 이식된 Matriderm® 및 주변 조직을 얻어 슬라이드로 제작하였다. 두께 변화, 혈관 형성, 콜라겐 및 탄력 섬유 합성 정도에 대하여 조직학적으로 관찰 및 분석하였다. 6mm 두께의 Matriderm® 은 4mm 두께의 Matriderm® 과 같은 정도 비율의 부피를 유지하였다. 40일의 실험 기간 동안 실험군, 대조군에서 두께가 감소하였지만, 이식 30일 이후에는 감소 정도가 둔화되어 두께가 의미있게 감소하지 않는 소견을 보였다. 혈관 형성, 콜라겐 및 탄력 섬유 합성은 수혜부 조직으로부터 가장 원위부에 위치한 중심선 부위에서 이식 초기에 원활하게 진행되었다. 콜라겐, 탄력 섬유는 시간이 흐름에 따라 점차 밀도가 증가하였으며, Matriderm® 은 새로 형성된 콜라겐, 탄력섬유로 대체되어 이식 40일 경과 후에 본래의 구조는 더 이상 유지되지 않았다. 현미경 소견 상 콜라겐 섬유는 건강한 정상 조직에서 관찰된 것과 같이 불규칙하게 분포 하였으며, 이것은 흉터 조직에서 관찰되는 규칙적 그리고 평행한 콜라겐 분포와는 반대되는 소견이었다. Matriderm® 은 조직 내성장이 원활하게 이루어지도록 도와주는 지지체 역할을 하는 것으로 판단되며, 연조직 보강을 위한 임상 적용이 가능할 것으로 사료 된다.

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**핵심되는 말: 콜라겐-엘라스틴 진피 대체물, 두께, 혈관 형성, 콜라겐 섬유, 탄력 섬유**