의학석사 학위논문

돼지에 이식한 포괄적인 항석회화 처리를 시행한 새로운 소 심낭 패치의 조기 성적

Early results of novel bovine pericardial patch using comprehensive anti-calcification procedure in a swine model

2016년 2월

서울대학교 대학원

의학과 흉부외과학 전공

최 재 웅

돼지에 이식한 포괄적인 항석회화 처리를 시행한 새로운 소 심낭 패치의 조기 성적

Early results of novel bovine pericardial patch using comprehensive anti-calcification procedure in a swine model

지도교수 임 청

이 논문을 의학석사 학위논문으로 제출함

2015년 10월

서울대학교 대학원

의학과 흉부외과학 전공

최 재 웅

최재웅의 의학석사 학위논문을 인준함

2015년 11월

위 원 장 _____ (인)

부위원장 _____ (인)

위 원 _____ (인)

Abstract

Early results of novel bovine pericardial patch using comprehensive anti-calcification procedure in a swine model

Jae-Woong Choi

Medicine

The Graduate School

Seoul National University

Introduction: Bovine pericardial patches have been widely used for various cardiovascular surgeries. However, calcification still remains an important drawback. We evaluated the short term safety and effectiveness of our comprehensive anti-calcification procedure in a comparative study of the novel and commercially available bovine pericardial patch in a swine implantation model.

Material and Methods: Our comprehensive anti-calcification procedure consisted of 4 steps, including decellularization with

sodium dodecyl sulfate and tritonX-100, space filler treatment with polyethylene glycol, glutaraldehyde cross-linking with organic solvent, and detoxification with glycine. We simultaneously implanted both the commercially available bovine pericardial patch (Supple Peri-Guard®) and novel bovine pericardial patch processed by the comprehensive anti-calcification procedure into the main pulmonary artery in 7 pigs. Every pig underwent a cardiac angiography and was humanely sacrificed on the 28th postoperative day. The extracted patches were stained with hematoxylin and eosin.

Results: All pigs survived for 4 weeks without any complication. Cardiac angiography showed the absence of leakage and structural problem. Neointimas were formed evenly without intimal hyperplasia. There were no significant differences in the degree of inflammation, necrosis, and calcification between the novel and commercially available patch($p = 0.450$, $p = 0.317$, $p = 0.999$).

Conclusions: Novel bovine pericardial patch using comprehensive anti-calcification procedure was similar to existing cardiovascular patch in early surgical results in a swine model. The comprehensive anti-calcification procedure could facilitate appropriate bioprosthetic properties of the bovine pericardium.

.....

Keywords: bovine pericardium, anti-calcification, bioprosthesis

Student Number: 2011-21866

목 차

List of figures and table

evenly with good endothelialization in (B) and (C). Among 7 cases, the one commercially available patch (CAP), which was observed with thrombus, was found with necrosis instead of endothelialization in (D). Grading Examples of NP in (E) and CAP in (F) (A:X10, B:X20, C:X40, D: X200, E,F:X100).

Table 1. Quantification of degree of inflammation, necrosis, and microcalcification

1. Introduction

Bovine pericardium is widely used for a variety of cardiovascular surgeries, as heart valve substitutes, intracardiac and vascular patch materials.[1] However, dystrophic calcification remains an important drawback of the xenograft. Therefore, there have been many studies to decrease calcification and improve the durability of xenograft.[2-5] We have been involved in the study of various issues of xenograft tissue engineering,[6-9] such as decellularization, space filler treatment, cross-linking, and detoxification. On the basis of these studies, we developed a comprehensive anti-calcification procedure and produced novel bovine pericardial patch using this technique.

In this study, the short term safety and effectiveness of our comprehensive anti-calcification procedure were evaluated in a comparative study of the novel and commercially available bovine

pericardial patch in a swine implantation model.

2. Material and Methods

The study protocol was reviewed and approved by the Institutional Animal Care and Use Committee of Seoul National University Bundang Hospital (IACUC No: BA1306-130/049-01, SNUBH animal research project No: 64-2013-049).

2.1. Materials

2.1.1. Bovine pericardial patches

This study was designed to compare the 2 bovine pericardial cardiovascular patches *i.e.* the novel patch (NP) and commercially available patch (CAP). The NP is a new complete product that is produced with a comprehensive anti-calcification procedure at a manufacturing facility that is certified for good manufacturing practice (GMP) (Figure 1). The size is 4 X 3cm and thickness is 0.3±0.1mm. The CAP was Supple Peri-Guard® made by Synovis Surgical Innovations and has been used for various surgical

procedures, including cardiac and vascular surgery. Our comprehensive anti-calcification procedure consisted of 4 steps, including decellularization with sodium dodecyl sulfate and tritonX-100, space filler treatment with polyethylene glycol, glutaraldehyde cross-linking with organic solvent, and detoxification with glycine. The comprehensive anti-calcification procedure is described below.

2.2 Methods

2.2.1 Comprehensive anti-calcification procedure

Fresh bovine pericardia obtained from a local slaughterhouse were placed in phosphate-buffered saline (PBS, pH 7.4) and immediately transported to our laboratory. All chemical processes were performed at the International Organization for standardization (ISO) class 5 and ISO class 7 cleanroom. After removing the excess fat and damaged tissue, they were rinsed with normal saline. Disinfection was achieved by treating the bovine pericardia

with phosphate-buffered saline (PBS, pH 7.3-7.5) containing 0.1% peracetic acid and 4% ethanol for 2 hours at 4℃. Sodium hydroxide was used for prevention of bovine spongiform encephalopathy (BSE), and propylene oxide was used for final sterilization. After completion of all process, aseptic condition was confirmed by sterility test.

(1) Decellularization

Decellularization was conducted in 4 steps. First, the tissues were treated with a hypotonic buffer solution that contained 0.25% sodium dodecyl sulfate (SDS) for 24hr and rinsed with washing solution for 1hr. Second, the tissues were treated with hypotonic buffer solution that contained 0.5% tritonX-100 for 24hr and washed with normal saline for 1hr. Third, they were treated with isotonic-buffered solution for 24hr and rinsed with washing solution for 1hr. Fourth, the tissues were treated with hypertonic-

buffered solution for 3hr and with PBS for 1hr. After decellularization, the tissues were preserved in PBS. All processes were performed at 4℃. The chemical composition of buffered solution and washing solution were as follows.

① Hypotonic buffered solution : distilled water 1,000mL; tris-HCL 0.01mol/L; ethylenediaminetetraacetic aicd (EDTA) disodium salt dihydrate 0.05%; neomycin trisulfate 50mg; pH 8.0;

② Isotonic buffered solution : distilled water 1,000mL; tris-HCL 0.05mol/L; NaCl 0.15mol/L; EDTA disodium salt dihydrate 0.05%; neomycin trisulfate 50mg; pH 8.0

③ Hypertonic buffered solution : distilled water 1000mL ; tris-HCL 0.1mol/L; NaCl 1.2mol/L; pH 8.0

④ Washing solution : distilled water 1,000mL; EDTA disodium salt dihydrate 0.05%; neomycin trisulfate 50mg

(2) Space filler treatment

Between the 3rd and 4th step of decellularization, the bovine pericardia were treated with PBS that contained 30% polyethylene glycol (PEG) 1000 molecular weight for 24hr at 4℃ to fill the interstitial space.

(3) GA cross-linking

Bovine pericardial tissues were cross-linked with 0.5% glutaraldehyde (GA) solution for 3 days and additionally cross-linked in 0.25% GA solution with 75% ethanol and 5% octanol for 2 days. Finally the tissues were fixed with 0.25% GA for 7 days. All processes were performed at room temperature.

(4) Detoxification

After the completion of GA cross-linking, the tissues were treated with PBS that contained 0.2M glycine for 24hr at room temperature.

2.2.2 Operation

We implanted the NP and CAP into the main pulmonary artery in 7

crossbred pigs (57.7±2.3 kg). The implantation sites were the proximal and distal part of the main pulmonary artery. The site was alternated between pigs.

Under general anesthesia, each pig was placed in the right lateral decubitus position and a left thoracotomy was performed through the 3rd intercostal space. On heparin injection, the proximal part of the pulmonary artery was partially clamped and excised. A cardiovascular patch was implanted using 5-0 polypropylene. The other cardiovascular patch was implanted on the distal part of the pulmonary artery in the same manner. Each patch was circular, with a 10mm diameter (Figure 2).

2.2.3 Postoperative care

Cefazolin and ketoprofen were injected postoperatively for 3 days and anticoagulants were not administered. Blood samples were taken from each pig on the operation day, the 1st, 7th, 14th, and 28th

postoperative days to measure complete blood cell count (CBC) and c-reactive protein (CRP). Postoperative care for the pigs was provided by our institution's veterinarian.

2.2.4 Cardiac angiography and histology

After heparin injection, the pigs were humanely sacrificed on the 28th postoperative day and the patches were harvested for macroscopic observation and histological study. Cardiac angiography was performed to evaluate the implanted patch areas prior to sacrificing the pig.

The extracted patches were stained with hematoxylin and eosin and observed for inflammation, necrosis, and microcalcification. We quantified each condition for subsequent statistical analyses; the grading was by our hospital's pathologist.

2.3 Statistical analysis

Statistical analyses were performed using the SPSS software

package (version 19.0, SPSS Inc., Chicago, IL). The data was expressed as mean ± standard deviation. Repeated measured analysis of variance (RM-ANOVA) was used to compare the changes in blood sample test and the Wilcoxon signed rank test was used to compare the changes in microscopic findings between the 2 patches. A p value < 0.05 was considered statistically significant.

3. Results

All 7 pigs survived for 4 weeks without any complication. All pigs maintained good food intake and weights increased from 57.7 ±2.3kg to 61.9±4.8kg.

3.1 Blood sample test

We checked CBC and CRP before and after surgery as a screening procedure for postoperative infection and other problems. Postoperative WBC and neutrophil counts showed a tendency to increase on the 7^{th} postoperative day and decrease on the 14^{th} and 28^{th} postoperative day. There were no significant differences in changes of WBC, neutrophil count and CRP value, respectively (p = 0.129, p = 0.128, p = 0.06, Figure 3).

3.2 Cardiac angiography and gross findings

Cardiac angiography demonstrated that there were no leakages or structural problems in the implanted patch areas. The blood flow of

main pulmonary artery was very smooth in all pigs (Figure 4).

There was no gross hematoma formation on the outer surface of the implanted patch areas. Two pigs developed observable thrombi on the inner surface of the implanted patches. One case was observed at a distal-located novel patch and the other was developed at a distal-located commercially available patch. The size was 4 x 2mm in NP and 7 x 5mm in CAP. The thrombus on the commercially available patch was approximately 4 times larger than the thrombus on the novel patch (Figure 5).

3.3 Histologic findings

The extracted patches were stained with hematoxylin and eosin in order to identify the degree of inflammation, necrosis, and microcalcification (Figure 6). All Inflammatory reactions were localized near the patches. Neointimas were formed evenly with good re-endothelialization except in one case. This one case was

in the CAP with an observed thrombus (Figure 6).

For quantification, we classified inflammation into 6 distinct grades according to infiltration extent of inflammatory cells; 0 = absent, 1 = minimal (infiltration extent ≤ 0.1mm), 2 = mild (0.1mm < infiltration extent ≤ 0.3mm), 3 = moderate (0.3mm < infiltration extent ≤ 1.0mm) and focal (one site), 4 = moderate (0.3mm < infiltration extent ≤ 1.0mm) and diffuse (more than one site), 5 = marked (1.0mm < infiltration extent). And necrosis was classified into 3 grades (0=absent, 1 = present< 50%, 2 = present≥ 50%), and microcalcification into 2 grades (0 = absent, 1= present). There were no significant differences in inflammation and necrosis between the 2 groups (p = 0.450, p = 0.317) and microcalcification was observed in all patches. Comprehensive evaluation of patch was performed by calculation of a total score that is sum of the inflammation, necrosis, and microcalcification grades in each patch.

The total score was not significantly different between the 2 patches (p = 0.581, Table 1).

Discussion

Xenograft tissues, such as bovine pericardium or porcine aortic valve, are widely used in the field of cardiovascular surgery. These xenografts require chemical processing to reduce immune or inflammatory reactions and to increase mechanical endurance for implantation in the human body. GA has been used as a representative cross-linking agent. However, GA cross-linking has known drawbacks, such as dystrophic calcification and cytotoxicity.

Based on our previous studies, we developed a comprehensive anti-calcification procedure using decellularization with sodium dodecyl sulfate and tritonX-100, space filler treatment with polyethylene glycol, GA cross-linking with organic solvent, and detoxification with glycine. Although the mechanisms of calcification of GA cross-linked xenograft are not fully

understood, the causes of calcification are known to be related to tissue phospholipids, free aldehyde groups of GA, and residual antigenicity.[10] Therefore, the comprehensive anti-calcification procedure targeted the removal of phospholipids and free aldehyde groups, as well as the reduction of the antigenicity of the xenograft.

Calcification of cardiac xenograft is initiated primarily within nonviable connective tissue cells that have been devitalized.[11] In this study, antigenicity was reduced by the decelluarization procedure that was performed in 4 steps using hypotonic solution, 0.25% SDS, and tritonX-100. We previously demonstrated that the stepwise use of SDS and tritonX-100 produced good decelluarization and a high concentration of SDS could cause structural alteration by compromising collagen integrity.[12,13] Using α-galactosidase in the decellularization process decreases the

immune response and prolongs durability of a xenograft. Despite our earlier reports on α-galactosidase treatment, [14-16] we were unable to use α-galactosidase in the comprehensive anti-calcification procedure because of low cost-effectiveness. The use of α-galactosidase for decelluarization would lead to a more effective anti-calcification procedure in the bovine pericardium.

Phospholipid is known to play an important role in calcification of the xenograft. Calcium-containing extracellular fluid is assumed to combine with phosphorus in the membrane associated phospholipid of dead xenograft cell membrane and form calcium phosphate crystals .[11] Treatment with organic solvents, such as ethanol, octanol, and octanediol, are known to reduce the phospholipid content and prevent calcium phosphate nucleation .[6] We used a short and long chain alcohol combination organic solvent. Pathak et al. reported that bovine pericardium treated

with a short- and long- chain alcohol combination shows a reduction in phospholipid content .[17] We further demonstrated the GA cross-linked bovine pericardium with a combination of short and long chain alcohol had a superior anti-calcification effect .[6] Our previous studies showed that GA and organic solvent treatment had better mechanical durability than GA treatment alone, and did not cause a loss in the tensile strength, elasticity, and thermostability .[18,19]

Amino groups can improve protein cross-linking and neutralize toxicity of free aldehyde groups .[20] Residual free aldehyde groups easily combine with serum Ca^{+} and result in tissue calcification.[21] Many studies showed that detoxification of GA cross-linked xenograft using various amino groups, such as homocysteic acid, L-Glutamic acid and L-arginine, was effective in reducing tissue calcification. We used glycine, the simplest form of all amino

acids, for detoxcification in the anti-calcification procedure. Our previous study demonstrated that post-fixation treatment with glycine strongly prevented calcification of GA fixed bovine pericardium in the rat subcutaneous implantation model.[8]

Our comprehensive anti-calcification procedure included space filler treatment using polyethylene glycol (PEG). Otherwise, the Supple Peri-Guard® used control group was not treated with space filler. Filling interstitial void spaces in GA treated tissue with macromolecular substance likely has a preventive effect on tissue calcification.[22] Although the mechanisms of space filler treatment are not fully understood, it is thought that reaction of macromolecules with free aldehyde groups of GA causes their inactivation and forms a barrier that prevents the release of residual GA.[23,24] We previously demonstrated that GA cross-linking with PEG as space filler was an effective anti-calcification

method in the rabbit intramuscular implantation model.[7]

Vasudev et al. reported that PEG substantially inhibited deposits of calcium and platelet-tissue attachment.[25] They hypothesized that PEG treatment modifies or masks the platelet receptor sites on collagen and reduces platelet density on the surface. It is thus likely that PEG treatment has not only an anti-calcification but also an anti-thrombogenic effect through prevention of platelet attachment. We thought that this property of PEG would cause the prevention of thrombus formation and thromboembolic events. The present study showed that the novel patch had smaller thrombus formation as compared to the commercially available patch that was not treated with a space filler.

This study had 4 main findings that indicated the safety and effectiveness of our comprehensive anti-calcification procedure. First, postoperative infection did not occur in all pigs. Second, we

could not find any structural problem related to the implanted patches. Third, neointima was evenly formed in the implanted novel patch. Fourth, there were no significant differences in the degree of inflammation, necrosis, and microcalcification between the 2 patches. These findings indicated that the novel patch was similar to the commercially available patch in terms of early surgical results in a swine model.

The present study had limitations that must be noted. First, the number of pigs enrolled in the present study might be too small and implantation period was too short to draw definite conclusions. Second, it is possible that the patch size was too small to affect structural changes, such as aneurysms or suture dehiscence. Furthermore, considering mechanical failure was exaggerated in high pressure systems, a definitive conclusion on the mechanical durability of the novel patch could not be reached. Third, our study

didn't reach to show the superiority on the novel patch. But, our comprehensive anticalcification procedure included the space filler treatement and showed the advantages in terms of endothelialization and thrombus formation. Therefore, we need a large sample and well designed long-term study to demonstrate more definitive effect of our comprehensive anti-calcification procedure.

Conclusions

Our comprehensive anti-calcification procedure was refined based on our previous studies. This study showed that novel bovine pericardial patch using comprehensive anti-calcification procedure was similar to existing cardiovascular patch in early surgical results in a swine model. Although thrombus formation was observed in both groups, the one on the CAP was approximately 4 times larger compared to the thrombus on the NP. Furthermore, the thrombus-formed CAP failed to achieve endothelialization. Therefore, our comprehensive anti-calcification procedure involving decellularization, space filler treatment, GA cross-linking, and detoxification was a good method to facilitate appropriate bioprosthetic properties of the bovine pericardium.

Reference

1. David TE: The use of pericardium in acquired heart disease: A review article. *J Heart Valve Dis* 1998, 7:13-18.

2. Carpentier A, Nashef A, Carpentier S, Ahmed A, Goussef N: Techniques for prevention of calcification of valvular bioprostheses. *Circulation* 1984, 70:1165-1168.

3. Vyavahare N, Hirsch D, Lerner E, Baskin JZ, Schoen FJ, Bianco R, Kruth HS, Zand R, Levy RJ: Prevention of bioprosthetic heart valve calcification by ethanol preincubation. Efficacy and mechanisms. *Circulation* 1997, 95:479-88.

4. Mathapati S, Bishi DK, Guhathakurta S, Cherian KM, Venugopal JR, Ramakrishna S, Verma RS: Biomimetic acellular detoxified glutaraldehyde cross-linked bovine pericardium for tissue engineering. *Mater Sci Eng C* 2013, 33:1561-1572.

5. Brizard CP, Brink J, Horton SB, Edwards GA, Galati JC,

Neethling WM: New engineering treatment of bovine pericardium confers outstanding resistance to calcification in mitral and pulmonary implantations in a juvenile sheep model. *J Thorac Cardiovasc Surg* 2014, 148(6):3194-201.

6. Chang HW, Kim SH, Kim KH, Kim YJ: Combined anti-calcification treatment of bovine pericardium with amino compounds and solvents. *Interact Cardiovasc Thorac Surg* 2011, 12:903-907.

7. Jeong S, Yoon EJ, Lim HG, Sung SC, Kim YJ: The effect of space fillers in the cross- linking processes of bioprosthesis. *Biores Open Access* 2013, 2:98-106.

8. Lee C, Kim SH, Choi SH, Kim YJ: High-concentration glutaraldehyde fixation of bovine pericardium in organic solvent and post-fixation glycine treatment: In vitro material assessment and in vivo anticalcification effect. *Eur J Cardiothorac Surg* 2011,

39:381-387.

9. Lim HG, Kim SH, Choi SY, Kim YJ: Anticalcification effects of decellularization, solvent, and detoxification treatment for genipin and glutaraldehyde fixation of bovine pericardium. *Eur J Cardiothorac Surg* 2012, 41:383-390.

10. Mathapati S, Bishi DK, Guhathakurta S, Cherian KM, Venugopal JR, Ramakrishna S, Verma RS: Biomimetic acellular detoxified glutaraldehyde cross-linked bovine pericardium for tissue engineering. *Mater Sci Eng C Mater Biol Appl 2013,* 33:1561-1572.

11. Schoen FJ, Levy RJ: Calcification of tissue heart valve substitutes: Progress toward understanding and prevention. *Ann Thorac Surg* 2005, 79:1072-1080.

12. Park CS, Kim Y-J, Sung S-C, Park JE, Choi S-Y, Kim W-H, Kim K-H: Study on effective decellularization technique for

xenograft cardiac valve, arterial wall and pericardium; optimization of decellularization. *Korean J Thorac Cardiovasc Surg* 2008, 41:550-562.

13. Jang WS, Kim YJ, Kim SH: Effects on tensile strength and elasticity after treatment with glutaraldehyde, solvent, decellularization and detoxification in fresh bovine pericardium. *Korean J Thorac Cardiovasc Surg* 2010, 43:1-10.

14. Park S, Kim WH, Choi SY, Kim YJ: Removal of alpha-gal epitopes from porcine aortic valve and pericardium using recombinant human alpha galactosidase a. *J Korean Med Sci* 2009, 24:1126-1131.

15. Choi SY, Jeong HJ, Lim HG, Park SS, Kim SH, Kim YJ : Elimination of alpha-gal xenoreactive epitope: Alpha-galactosidase treatment of porcine heart valves. *J Heart Valve Dis* 2012, 21:387-397.

16. Lee C, Ahn H, Kim SH, Choi SY, Kim YJ: Immune response to bovine pericardium implanted into alpha1,3-galactosyltransferase knockout mice: Feasibility as an animal model for testing efficacy of anticalcification treatments of xenografts. *Eur J Cardiothorac Surg* 2012, 42:164-172.

17. Pathak CP, Adams AK, Simpson T, Phillips RE, Jr., Moore MA: Treatment of bioprosthetic heart valve tissue with long chain alcohol solution to lower calcification potential. *J Biomed Mater Res A* 2004, 69:140-144.

18. Chang HW, Kim Y-J, Kim SH, Park JE, Park CS, Kim W-H, Kim K-H: The dynamic and histologic changes of variously fixed bovine pericardiums specimens after mechanical fatigue stimuli. *Korean J Thorac Cardiovasc Surg* 2009, 42:148-156.

19. Cho S, Kim Y-J, Kim SH, Park JE, Kim W-H: Comparison of the uniaxial tensile strength, elasticity and thermal stability

between glutaraldehyde and glutaraldehyde with solvent fixation in xenograft cardiovascular tissue. *Korean J Thorac Cardiovasc Surg* 2009, 42:165-174.

20. Jorge-Herrero E, Fernández P, Escudero C, García-Páez JM, Castillo-Olivares JL: Calcification of pericardial tissue pretreated with different amino acids. *Biomaterials* 1996, 17:571-575.

21. Zilla P, Weissenstein C, Bracher M, Zhang YX, Koen W, Human P, vonOppell U: High glutaraldehyde concentrations reduce rather than increase the calcification of aortic wall tissue. *J Heart Valve Dis* 1997, 6:502-509.

22. Oosthuysen A, Zilla PP, Human PA, Schmidt CA, Bezuidenhout D: Bioprosthetic tissue preservation by filling with a poly(acrylamide) hydrogel. *Biomaterials* 27:2123-2130, 2006.

23. Chanda J: Anticalcification treatment of pericardial prostheses. *Biomaterials* 1994, 15:465-469.

24. Chanda J, Kuribayashi R, Abe T: Use of the glutaraldehyde-chitosantreated porcine pericardium as a pericardial substitute. *Biomaterials* 1996, 17:1087-1091.

25. Vasudev SC, Chandy T, Sharma CP: The antithrombotic versus calcium antagonistic effects of polyethylene glycol grafted bovine pericardium. *J Biomater Appl* 1999, 14:48-66.

Figure 1. A: Gross appearance of novel patch (3 X 4 cm). B: Microsopic view of novel patch.

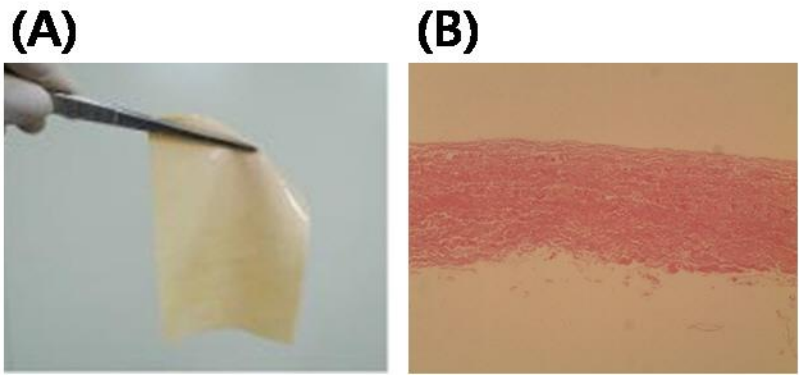


Figure 2. Main pulmonary artery state after patch implantation.

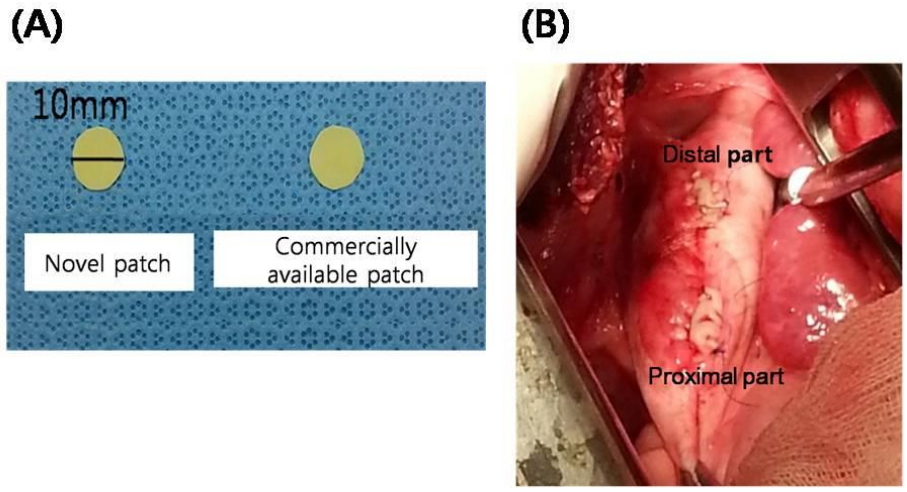


Figure 3. Results of blood sample test. POD, postoperative day; WBC, white blood cell; CRP, c-reactive protein

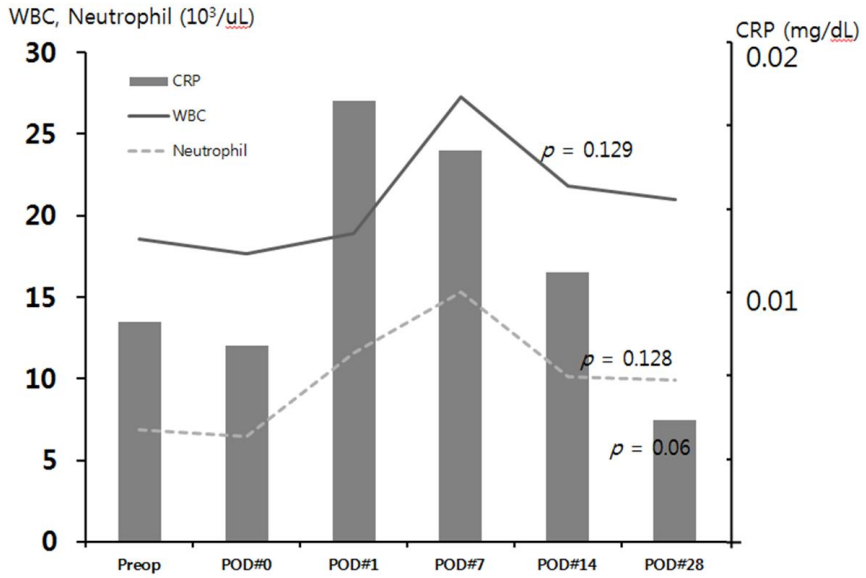


Figure 4. Results of cardiac angiography. Blood flow was very smooth and there was no structural problem. MPA, main pulmonary artery

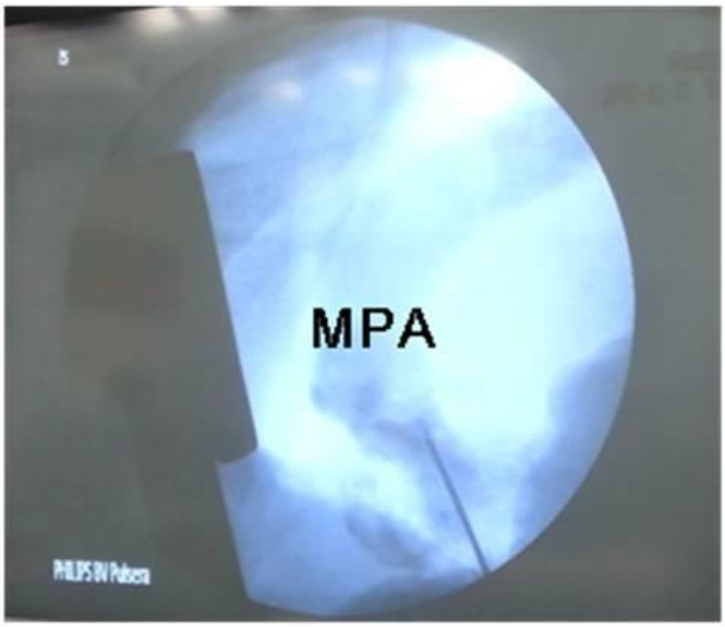


Figure 5. Gross findings of extracted pulmonary artery in (A) and (B). There was no gross hematoma formation on the outer surface. Thrombus were observed at distal located NP and distal located commercially available patch (CAP) in (C) and (D). The thrombus on the CAP was approximately 4 times larger than the thrombus on the NP.

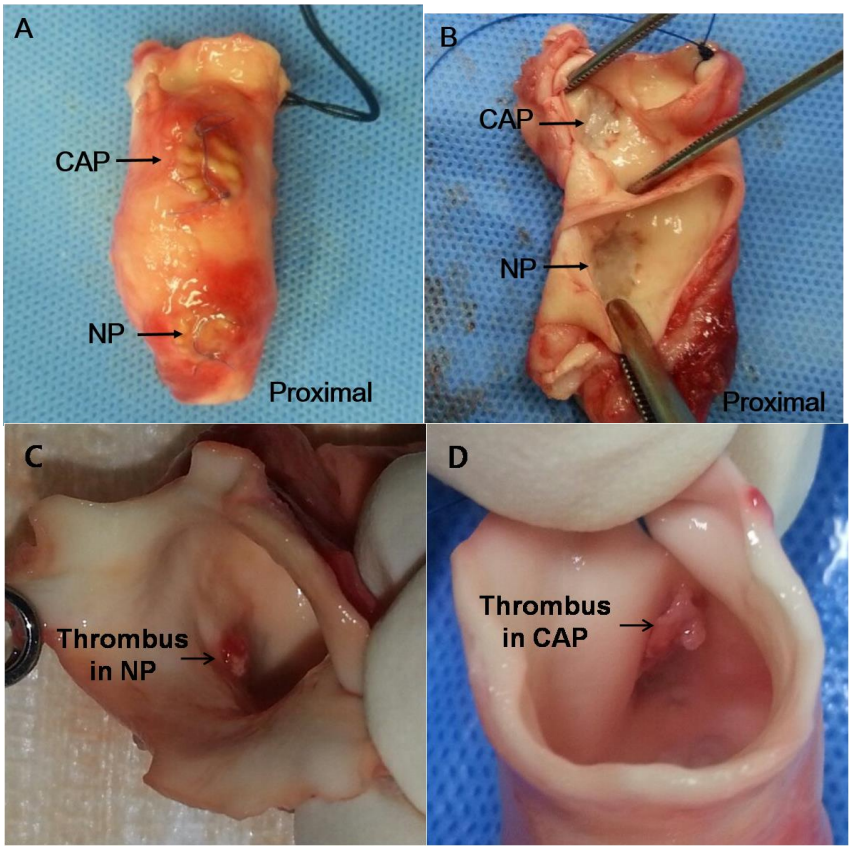
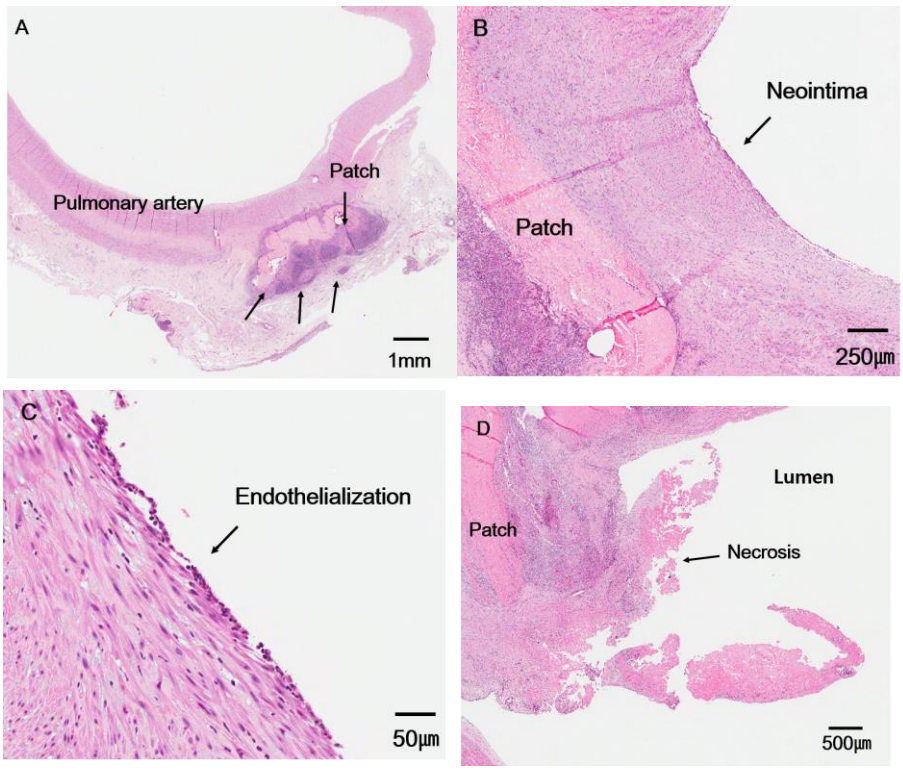


Figure 6. Microscopic views of extracted patches. Inflammation was restricted around the patch in (A). Neointima was formed evenly with good endothelialization in (B) and (C). Among 7 cases, the one commercially available patch (CAP), which was observed with thrombus, was found with necrosis instead of endothelialization in (D). Grading Examples of NP in (E) and CAP in (F) (A:X10, B:X20, C:X40, D: X200, E,F:X100).



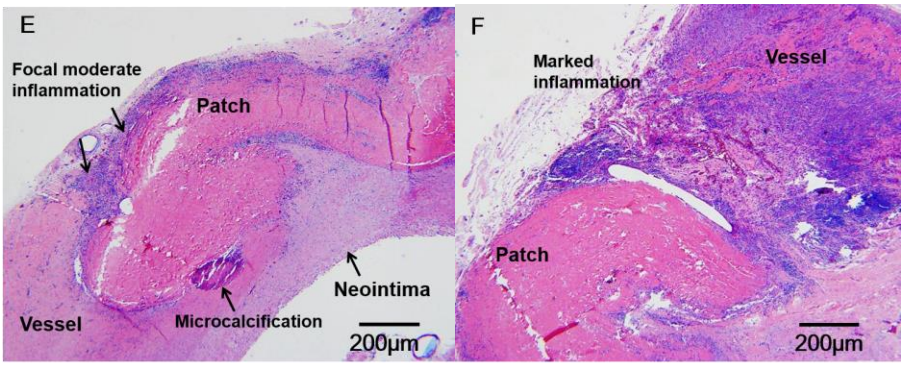

E
Focal moderate inflammation
Patch
Neointima
Microcalcification
Vessel
200μm
F
Marked inflammation
Vessel
Patch
200μm

Table 1. Quantification of degree of inflammation, necrosis, and microcalcification

		No1	No2	No3	No4	No5	No6	No7	Total	*p*
Inflam-	NP	3	3	5	3	5	5	5	4.14±1.07	
										0.450
mation	CAP	5	5	5	4	3	5	5	4.57±0.79	
	NP	0	1	0	0	0	1	1	0.43±0.53	
Necrosis										0.317
	CAP	0	1	0	0	0	1	0	0.29±0.49	
Microcal-	NP	1	1	1	1	1	1	1	1.0	
										>0.999
Cification	CAP	1	1	1	1	1	1	1	1.0	
Total	NP	4	5	6	4	6	7	7	5.57±1.27	
										0.581
score	CAP	6	7	6	5	4	7	6	5.86±1.07	

NP, Novel patch; CAP, Commercially available patch

Inflammation grades; 0 = absent; 1 = minimal (infiltration extent ≤ 0.1mm), 2 = mild (0.1mm < infiltration extent ≤ 0.3mm), 3 = moderate (0.3mm < infiltration extent ≤ 1.0mm) and focal (one

site), 4 = moderate (0.3mm < infiltration extent ≤ 1.0mm) and diffuse (more than one site), 5 = marked (1.0 < infiltration extent)

Necrosis grades; 0 = absent, 1 = present < 50%, 2 = present ≥ 50%

Microcalcification grades; 0 = absent, 1 = present

국문초록

돼지에 이식한 포괄적인 항석회화 처리를 시행한 새로운 소 심낭 패치의 조기 성적

서울대학교 대학원

의학과 흉부외과학 전공

최 재 웅

서론: 소 심낭 패치는 다양한 심혈관 수술에 널리 이용되지만 석회화는 아직까지 중요한 문제점으로 남아있다. 이에 돼지에 이식한 상품화 되어있는 소 심낭 패치와 새로운 소 심낭 패치의 비교를 통해 포괄적인 항석회화 처리의 조기 안정성과 효과를 평가해 보고자 하였다.

방법: 포괄적 항석회화 처리는 sodium dodecyl sulfate 와 tritonX-100을 이용한 탈세포화, polyethylene glycol을 이용한 space filler treatment, glutaraldehyde와 유기 용제를 이용한 고정, glycine을

이용한 detoxification을 포함한 4 단계로 구성되어 있다. 상품화 되어 있는 소 심낭 패치(Supple Peri-Guard)와 포괄적 항석회화 처리를 통해 생산된 새로운 소 심낭 패치를 각각의 7마리의 돼지의 주폐동맥에 동시에 이식하였다. 수술 후 28일째 모든 돼지는 심장혈관 조영술 시행 후 안락사 시켰다. 안락사 후 추출된 패치는 hematoxylin and eosin (H&E) 염색을 시행하였다.

결과: 모든 돼지는 특별한 합병증 없이 4주간 생존하였다. 심장혈관 조영술을 통해 이식 부위의 누출이나 구조적인 문제가 없음을 확인할 수 있었으며, H&E 염색을 통해 신생혈관내막이 증식 없이 고르게 형성된 것을 확인할 수 있었다. 두 패치간 염증과 괴사, 석회화 정도를 비교하였을 때 유의한 차이를 보이지 않았다 ($p = 0.450$, $p = 0.317$, $p = 0.999$).

결론: 포괄적 항석회화 처리를 이용한 새로운 소 심낭 패치는 돼지 이식 모델에서 기존의 패치와 유사한 조기 성적을 보였다. 포괄적 항석회화 처리는 소 심낭이 적합한 생체인공삽입물의 특성을 갖도록

촉진하는 것으로 생각된다.

……

주요어: 소 심낭, 항석회화, 생체인공삽입물

학 번: 2011-21866