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Calcification Resistance of Procyanidin-Treated Decellularized Porcine Aortic Valves In Vivo

Yang Liu, Weiyong Liu, Guocheng Sun, Xufeng Wei, Dinghua Yi

Department of Cardiac Surgery, Xijing Hospital, Forth Military Medical University, Xi'an, China

ABSTRACT

Objectives: Conventional glutaraldehyde fixation is conducive to calcification of bioprosthetic tissues. The aim of this study was to test calcification resistance of procyanidin-treated decellularized porcine aortic valve in a rat model.

Materials and methods: We performed cross-linking of the decellularized porcine aortic heart valves by procyanidins and observed morphologic performance and examined the tensile strength and cross-linking index. Then we implanted subcutaneous samples of procyanidin cross-linking decellularized valves, glutaraldehyde cross-linking decellularized valves, and decellularized valves in rats. The retrieved grafts were stained with hematoxylin–eosin and von Kossa and were analyzed with scanning electron microscopy and x-ray energy dispersive spectroscopy (EDS) after 21 and 63 days.

Results: After decellularized and cross-linking pretreatment, the procyanidin cross-linked leaflets were soft and stretchable. In addition, the cellular components of the porcine aortic heart valve leaflets were completely removed, and the extracelluar matrix was maintained completely. Examination of tensile strength revealed a significantly higher tissue resistance to tension in procyanidin cross-linked tissue than in other tissues, including the glutaraldehyde group ($P < .05$), even though the extents of cross-linking of each group were roughly the same at approximately 90%. Histopathologic examination showed that the procyanidin cross-linked valve matrix had no significant calcification, and there were no calcium peaks in the EDS profile of procyanidin cross-linked samples in the 21-day and 63-day rat studies.

Conclusion: This study demonstrated that procyanidin cross-linked decellularized heart valves can resist calcification to some extent.

INTRODUCTION

Heart valve diseases have significantly high mortality. More than 275,000 prosthetic cardiac valves are implanted all over the world annually [Simionescu 2004]. Mechanical and xenogeneic bioprosthetic (tissue) valves are 2 primary choices for heart valve substitutes. The key drawbacks to mechanical valves include the need for life-long anticoagulation therapy, bleeding disorders, and flow dynamics that are distinctly different from those of normal heart valves [Zilla 2008]. Although xenografts have many advantages, including an unlimited supply of donor tissue, superior hemodynamics, and fewer thromboembolic complications compared to mechanical valves, tissue-based valves tend to have shorter lifetimes owing to complications with calcification and leaflet wear [Schoen 2005]. Previous studies have demonstrated that conventional glutaraldehyde fixation is conducive to calcification of bioprosthetic tissues [Schinke 1999; Schoen 1999b]. Therefore, other studies have investigated modifications of and alternatives to conventional glutaraldehyde pretreatment [Schoen 1999a] and fixation of bioprosthetic tissue (cusps and aortic wall) with other cross-linking reagents such as aminooleic acid, ethanol, epoxy compounds, carbodiimides, and acyl azide [Nemes 1985; Broyn 1986; Ferrans 1991; Schmitz-Rixen 1991; Park 1994; Naimark 1995; Girardot 1996]. None of these tissue fixatives, however, met the requirements for the preparation of the heart valve bioprosthesis.

According to previous studies, cells are important in the process of inflammation and mineralization of implants [Pereira 1990]. The mineralization process of bioprosthetic heart valves is initiated predominantly within nonviable connective tissue cells that have been devitalized but not removed by pretreatment procedures [Schmidt 2000]. These investigations indicate that decellularization approaches may reduce host immune response to bioprosthetics and make the bioprosthetic matrix less prone to calcification. However, the use of this approach on clinically implanted nonfixed homografts has yielded unfavorable results [Bechtel 2003; Simon 2003]. The potential reason may be inferior enzymatic degradation resistance and mechanical performance attributable to the lack of cross-linking pretreatment.

Procyanidins are particularly important polyphenols as complements to the organism's antioxidant defense system. Procyanidins are potent free-radical scavengers and are preventive against cardiovascular diseases, cancer, and other disorders; they are increasingly used as natural food additives, acting as flavoring, coloring, and antioxidant agents [Plumb 1998]. In addition to these bioactivities, Han et al. have reported that procyanidins can be used as a reagent for cross-linking collagen materials [Han 2003]. Another study indicated that procyanidins have low toxicity, and when used as a cross-linking reagent they enhanced the mechanical tensile strength, stability, and durability of the porcine heart valves [Zhai 2006]. The anticalcific performance of heart

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Correspondence: Weiyong Liu, Department of Cardiac Surgery, Xijing Hospital, Forth Military Medical University, Xi'an Shaanxi 710032, China; 86-29-84775311 (e-mail:weiyongl@foxmail.com).

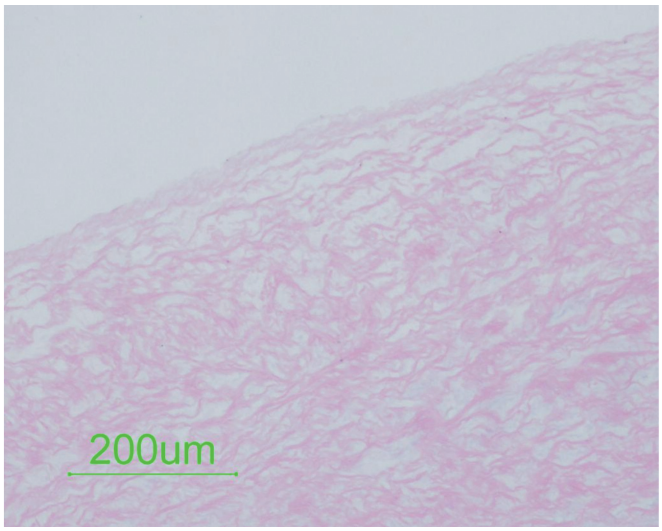

Figure 1. Representative histomicrographs of procyanidin-treated decellularized porcine aortic valve tissue. (Hematoxylin–eosin, original magnification ×200).

valves with procyanidin cross-linking has not been reported. Therefore, we tested calcification resistance of procyanidin-treated decellularized porcine aortic valves in a rat model.

MATERIALS AND METHODS

Decellularization Process

Fresh porcine aortic heart valves procured from a local slaughterhouse were used as raw materials. After removal of adherent fat, the porcine valves were stored in phosphate-buffered solution at 4°C. The procedure used to remove the cellular components from porcine aortic heart valves was based on a previously reported method [Cebotari 2002]. Briefly, porcine aortic heart valves were incubated in a D-Hanks solution with 0.5% triton X-100, 0.5% sodium deoxycholate, and 0.02% EDTA for 48 hours. Then the valves were incubated with ribonuclease A (20 μg/mL) and deoxyribonuclease (0.2 mg/mL) for 2 hours to remove cellular components. After thorough rising with phosphate buffered saline (PBS), the decellularized valves were stored in D-Hanks solution at 4°C.

Fixation Process

The decellularized valves were cross-linked by soaking in procyanidins (a generous gift of Xi'an Guanyu Natural Product R&D, Xian, China) solution with a concentration of 10 mg/mL in D-Hanks solution (pH 7.4) at 37°C under continuous shaking. Control valves were cross-linked by 6.25 mg/mL glutaraldehyde under the same conditions.

Morphologic Observations

The cross-linked decellularized valves were fixed in 10% formalin solution and embedded in paraffin. Vertical cross sections of 5-μm thickness were obtained and stained with hematoxylin–eosin. At the same time, the valves were observed by scanning electron microscopy (SEM). The specimens were fixed by immersion in glutaraldehyde (2.5%, in D-Hanks solution) for 4 hours. We then washed the specimens 3 times with PBS solution, followed by dehydration in ascending concentrations of ethanol (30%, 50%, 70%, 90%, 95%, 100% [volume/volume]) for 10 minutes each. The specimens were dried with a 50% alcohol-hexamethyldisilazane solution (volume/volume) for 10 minutes, then in pure hexamethyldisilazane for 10 minutes and finally air dried in a desiccator overnight. The dried specimens were sputter-coated with gold before observation.

Tensile Strength

To examine the tensile strength of the cross-linked decellularized valve leaflets, each leaflet from an individual group was cut along the collagen fiber direction to yield 1 strip with a width of 4 mm and a length of 25 mm. A Shimadzu (Kyoto, Japan) material testing instrument at a 2 mm/min extension rate was used to measure the tensile strength of 6 tissue straps in each group.

Evaluation of Cross-Linking Index

The extent of cross-linking is indicated by the cross-linking index defined as:

$$\textbf{cross-linking index (\%)} = (NH_0 - NHt)/NH_0 \times 100$$

where NH_0 is the amount of free amino groups in the scaffold before cross-linking and NH_t is the amount of free amino groups in the scaffold after cross-linking.

The amount of free amino groups was determined by the Ninhydrin assay associated with an enzyme-linked immunoassay reader (Tecan, Männedorf, Switzerland).

Rat Implantation

Three different treated valve materials, procyanidin cross-linking decellularized, glutaraldehyde cross-linking decellularized, and decellularized valves, were implanted in rats. Samples in each treatment group were sterilized by irradiation with ^{60}Co and color coded with a surgical suture before implantation. Rat studies were performed in compliance with

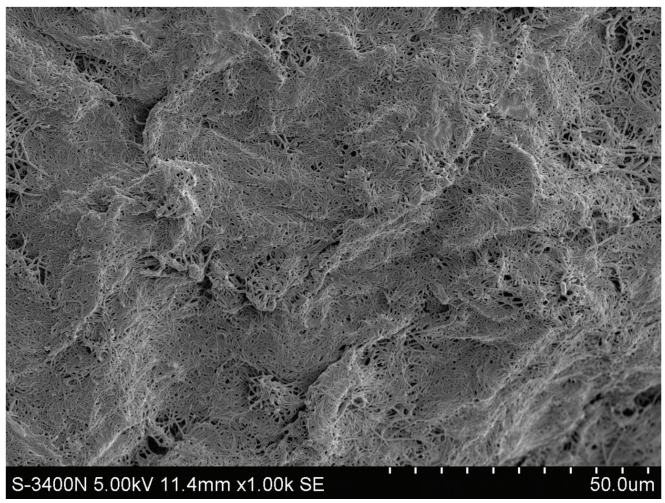

Figure 2. Scanning electron microscope micrographs of decellularized porcine aortic valve treated with procyanidins.

the animal care committee guidelines for small animal operations. Three-week-old male Sprague Dawley rats were used. They received a preoperative injection of ketamine hydrochloride 85 mg/kg by intraperitoneal injection. An incision was made in the back of each rat, and samples of the 3 treatment groups were implanted into individual subcutaneous pockets. At the termination of the study (21 and 63 days) animals were killed with intraperitoneal injection of Beuthanasia solution (Schering-Plough, Union, NJ, USA), 86 mg/kg mixed 1:1 with normal saline. Explanted samples were placed in 0.9% saline for storage before analysis. Each tissue sample was removed from the saline and sectioned in half. One-half of the tissue sample was placed in 10% neutral buffered formalin and stored for histologic examination. The second half of the tissue was cleaned of host tissue and used for SEM x-ray energy dispersive spectroscopy (EDS) analysis.

Histology

The retrieved grafts were fixed in 10% formalin solution and embedded in paraffin. Vertical cross sections of 5-μm thickness were obtained and treated with hematoxylin–eosin and von Kossa staining.

SEM-EDS Analysis

The retrieved grafts cleaned of host tissue were analyzed with SEM-EDS (LEO 440i; Leo Electron Microscopy, Cambridge, UK).

Statistics

All data are reported as mean ± SD. The unpaired Student *t*-test was used for analyses. Statistical significance was defined as $P < .05$. The SPSS statistical software package 13.0 for Windows (SPSS) was used for statistical analysis.

RESULTS

Preparation of Cross-Linking Decellularized Valves

The color of decellularized valve leaflets cross-linked by procyanidins changed to a slight crimson. The procyanidin cross-linked leaflets were soft and stretchable whereas the glutaraldehyde cross-linked leaflets were rigid and slightly contracted. After decellularizing and cross-linking pretreatment, the cellular components of the porcine aortic heart valve leaflets were completely removed, and the extracelluar matrix was maintained completely (Figures 1 and 2).

Tensile Strength

The valve matrix tensile strengths were 21.7 ± 2.1 MPa (n = 6) for procyanidin cross-linked, 10.9 ± 3.4 MPa (n = 6) for glutaraldehyde cross-linked, 9.7 ± 1.7 MPa (n = 6) for non–cross-linked decellularized, and 9.3 ± 1.4 MPa (n = 6) for native valve leaflets. These results are shown in Figure 3. Examination of tensile strength revealed significantly higher tissue resistance to tension in procyanidin cross-linked tissue than in others ($P < .05$).

Extent of Cross-Linking

The extent of cross-linking of the cross-linked decellularized valves prepared with procyanidins and glutaraldehyde is shown in Figure 4. The extent of cross-linking of each group was approximately the same, at about 90%. (The extent of cross-linking of the procyanidin cross-linked valve matrix was 90.7 ± 6.1% [n = 6], and the extent of cross-linking of glutaraldehyde cross-linked valve matrix was 89.3 ± 7.6% [n = 6]). There were no significant differences in the degree of cross-linking between the procyanidin cross-linked and glutaraldehyde cross-linked valve matrix.

Histological Evaluation

Histopathologic examination of the valves from the 21-day rat study indicated that the greatest degree of calcification was in the glutaraldehyde cross-linked valve matrix. The degree of calcification in the procyanidin cross-linked valve matrix was similar to the decellularized group, which showed no significant calcification (Figure 5). In the 63-day rat study, the greatest degree of calcification occurred in the glutaraldehyde cross-linked valve matrix, followed by the decellularized group, whereas the procyanidin cross-linked valve matrix showed no significant calcification (Figure 5). Calcification of each group was localized primarily in the interface between the valve and host tissue.

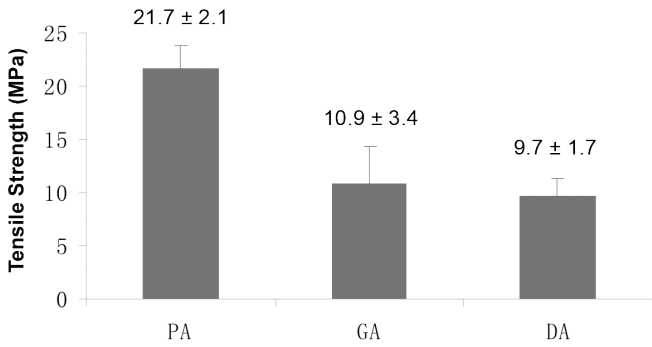

Figure 3. The tensile strength of procyanidin cross-linking decellularized valves (PA), glutaraldehyde cross-linking decellularized valves (GA), and decellularized valves (DA). The tensile strength of valves cross-linked by procyanidins was higher than that of valves that were non–crosslinked or crosslinked by glutaraldehyde; *$P < .05$.

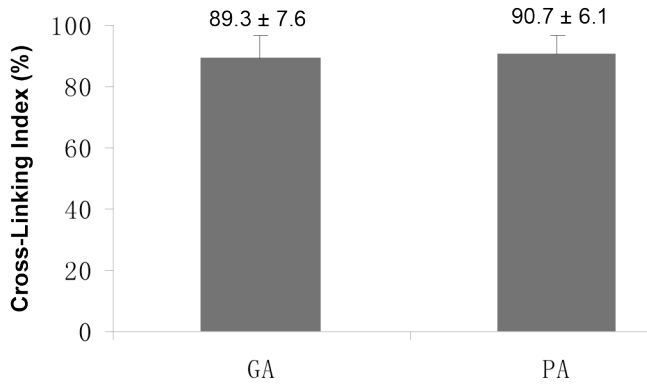

Figure 4. The cross-linking index of procyanidin cross-linking decellularized valves (PA) and glutaraldehyde cross-linking decellularized valves (GA). There were no significant differences; $P > .05$.

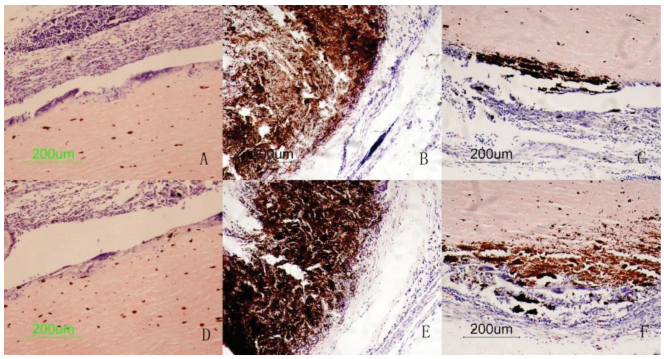

Figure 5. Histomicrographs showing calcium distribution in bioprosthetic rat explants. (A) Procyanidin cross-linking decellularized valves in the 21-day rat study. (B) Glutaraldehyde cross-linking decellularized valves in the 21-day rat study. (C) Decellularized valves in the 21-day rat study. (D) Procyanidin cross-linking decellularized valves in the 63-day rat study. (E) Glutaraldehyde cross-linking decellularized valves in the 63-day rat study. (F) Decellularized valves in the 63-day rat study. (Von Kossa stain, original magnification ×200).

EDS Analysis

Figure 6 shows the EDS profile of explants from the 21-day and 63-day rat studies. There are no calcium peaks in the EDS profile of procyanidin cross-linked samples either the 21-day or the 63-day rat study. The EDS profile indicates that the greatest degree of calcium deposits occurred in the glutaraldehyde cross-linked valve matrix after being embedded subcutaneously for 21 and 63 days. The calcium peaks are more significant for 63-day than the 21-day rat study. In the decellularized group, calcium peaks are absent in 21-day rat study, whereas very low calcium peaks are present in the 63-day rat study.

DISCUSSION

Because of the multiple shortcomings of conventional prostheses, valve replacement is usually done with bioprosthetic heart valves. The production of currently used bioprostheses requires glutaraldehyde fixation, which in itself has been shown to contribute to degeneration [Bernacca 1994; Mako 1997]. Therefore, various anticalcification treatment protocols have been developed to improve durability [Pettenazzo 2001].The mineralization process in the cusps of bioprosthetic heart valves is initiated predominantly within nonviable connective tissue cells that have been devitalized but not removed by glutaraldehyde pretreatment procedures [Levy 1983; Schoen 1985; Valente 1985; Schoen 1986]. This dystrophic calcification mechanism involves reaction of calcium-containing extracellular fluid with membrane-associated phosphorus, causing calcification of the cells. Therefore, in this study we used 2 main pretreatment procedures, decellularization and procyanidin cross-linking.

Before being cross-linked by procyanidins, we first obtained a cell-free valve matrix by a decellularization process. The results of this study indicate that decellularization can contribute to anticalcification of valves. Decellularized valves are always used as the scaffold material for tissue-engineered heart valves. Decellularization methods have been pursued as a means to create completely acellular tissues for use as biomaterial implants, because residual cellular components and lipids within processed tissue may promote calcification [Courtman 1994; Wilson 1995; Schmidt 2000; Schoen 2005]. For tissue engineering purposes decellularization is considered the method of choice to reduce or eliminate antigenicity, whereas for bioprosthetic valves decellularization approaches can be used to remove the calcinuclea in addition to reducing antigenicity. In this study, the cellular components of the porcine aortic heart valve leaflets were completely removed, and the extracelluar matrix was maintained completely (Figures 1 and 2) after decellularization pretreatment. Histopathologic examination showed significantly less calcification in the decellularized than in the glutaraldehyde cross-linked valve matrix in the 21-day and 63-day rat studies. Additionally, the EDS profile showed that in the decellularized group calcium peaks were absent in the 21-day rat study, whereas the very low calcium peaks were present in 63-day rat study. These results indicated that decellularization treatment of the bioprosthetic matrix lowers immunogenicity and makes the material less prone to calcification, similarly to results of previous studies.

These effects of decellularization are not sufficient, however. The use of natural biomaterials has typically required chemical or physical pretreatment aimed at preservation by enhancing the resistance of the material to enzymatic or chemical degradation, reduction of immunogenicity, and sterilization [Rapoport 2007]. Early failure of the SynerGraft (Cryolife; Kennesaw, GA, USA) indicated that decellularized valves required additional treatment [Bechtel 2003; Simon 2003]. Although cross-linking reagents may be the appropriate treatment choice, many new cross-linking methods expected to replace glutaraldehyde pretreatment were not

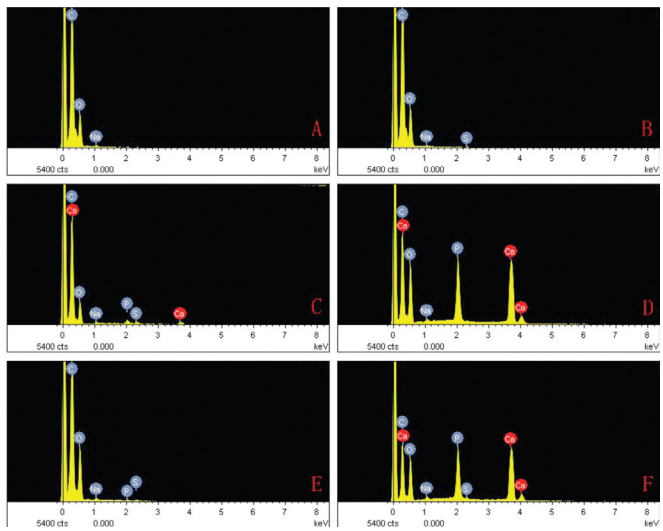

Figure 6. EDS spectrum for bioprosthetic rat explants. (A) Procyanidins crosslinking decellularized valves in the 21-day rat study. (B) Procyanidins crosslinking decellularized valves in the 63-day rat study. (C) Glutaraldehyde crosslinking decellularized valves in the 21-day rat study. (D) Glutaraldehyde crosslinking decellularized valves in the 63-day rat study. (E) Decellularized valves in the 21-day rat study. (F) Decellularized valves in the 63-day rat study.

ideal because of problems with mechanical tensile strength, stability, and durability. In this study we chose procyanidin, a new cross-linking reagent with low toxity that enhances the mechanical tensile strength, stability, and durability of animal heart valves [Han 2003; Zhai 2006].

Procyanidins are particularly important polyphenols because they are complements to the organism's antioxidant defense system [Packer 1999]. Procyanidins have many bioactivitive functions [Shahat 2002; Aldini 2003; Jayaprakasha 2003]. Unlike glutaraldehyde, which reacts with the ε-amino group of lysine residues in proteins and forms a covalent bond, procyanidin cross-linking involves the formation of hydrogen bonds between the phenolic hydroxyl and the matrix protein amide carbonyl [Zhao 1999; Dongmo 2001]. In this study, examination of tensile strength revealed significantly higher tissue resistance to tension in procyanidin cross-linked tissue than in other treated tissues, including the glutaraldehyde group ($P < .05$), even though the extent of cross-linking of each group of treated tissues we tested was roughly the same, at about 90%. These results indicated that procyanidin is superior to glutaraldehyde in enhancing mechanical strength. On the other hand, histopathologic examination showed that the procyanidin cross-linked valve matrix had no significant calcification in the 21-day and 63-day rat studies. There were no calcium peaks in the EDS profile of the procyanidins cross-linked samples the 21-day and 63-day rat studies. Our results showed that the procyanidin-fixed porcine heart valve matrix significantly resisted calcification in a rat model, a result that was comparable to valves that were cross-linked by glutaraldehyde or decellularized.

The anticalcification performance of procyanidins may be associated with their effect on lipid metabolism [Ogino 2007; Sugiyama 2007]. Cell membranes and other intercellular structures are high in phospholipids, which can bind calcium and serve as nucleators. Thus removal of cellular elements and reduction of lipid content are very important factors for preventing calcification. In this study, we used decellularization to remove cellular content and then cross-linked the decellularized valve matrix with procyanidins. Histopathologic examination indicated that the procyanidin cross-linked valve matrix had no significant calcification in the 21-day and 63-day rat studies, whereas only decellularized valves showed slight calcification in the 21-day and 63-day rat studies. EDS profiles showed the similar results, with calcium peaks in the 63-day rat study occurring only in the decellularized group.

Our results suggest that the procyanidin cross-linking process could have a beneficial effect on lipid removal in the valve matrix, but this hypothesis was not evaluated in this study and further studies would be required.

Although this study demonstrated significant anticalcification in the procyanidins cross-linked decellularized valves in vivo, there are many challenges to widespread application of procyanidins for tissue graft treatment. Further purification of the active cross-linking is needed, and optimal cross-linking conditions need more investigation. The cross-linking matrix performance may vary under conditions with differing Ph, temperature, and concentrations. Long-term durability of the procyanidin cross-linking decellularized valve is another important problem that requires investigation. The present subdermal studies in rats must be confirmed in experimental circulatory implants, such as sheep aortic or mitral valve replacements, to confirm that the hemodynamic and anticalcification processes are active and effective in the blood-material environment.

CONCLUSION

This study demonstrated that procyanidin cross-linked decellularized heart valves can resist calcification to some extent. These results suggest that decellularization and procyanidins might be new methods to prepare bioprosthetic heart valve xenografts.

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REFERENCES

Aldini G, Carini M, Piccoli A, Rossoni G, Facino RM. 2003. Procyanidins from grape seeds protect endothelial cells from peroxynitrite damage and enhance endothelium-dependent relaxation in human artery: new evidence for cardio-protection. Life Sci 73:2883-98.

Bechtel JF, Muller-Steinhardt M, Schmidtke C, Bruswik A, Stierle U, Sievers HH. 2003. Evaluation of the decellularized pulmonary valve homograft (SynerGraft). J Heart Valve Dis 12:734-9.

Bernacca GM, Tobasnick G, Wheatley DJ. 1994. Dynamic in-vitro calcification of porcine aortic valves. J Heart Valve Dis 3:684-7.

Broyn T, Christensen O, Fossdal JE, Kordt KF, Kroese A, Myhre HO. 1986. Early complications with a new bovine arterial graft (Solcograft-P). Acta Chir Scand 152:263-6.

Cebotari S, Mertsching H, Kallenbach K, et al. 2002. Construction of autologous human heart valves based on an acellular allograft matrix. Circulation 106(12 suppl 1):I-63-8.

Courtman DW, Pereira CA, Kashef V, McComb D, Lee JM, Wilson GJ. 1994. Development of a pericardial acellular matrix biomaterial: biochemical and mechanical effects of cell extraction. J Biomed Mater Res 28:655-66.

Dongmo AB, Kamanyi A, Anchang MS, et al. 2001. Anti-inflammatory and analgesic properties of the stem bark extracts of Erythrophleum suaveolens (Caesalpiniaceae) Guillemin & Perrottet. J Ethnopharmacol 77:137-41.

Ferrans VJ, Milei J, Ishihara T, Storino R. 1991. Structural changes in implanted cardiac valvular bioprostheses constructed of glycerol-treated human dura mater. Eur J Cardiothorac Surg 5:144-54.

Girardot JM, Girardot MN. 1996. Amide cross-linking: an alternative to glutaraldehyde fixation. J Heart Valve Dis 5:518-25.

Han B, Jaurequi J, Tang WB, Nimni ME. 2003. Proanthocyanidin: a natural crosslinking reagent for stabilizing collagen matrices. J Biomed Mater Res 65:118-24.

Jayaprakasha GK, Selvi T, Sakariah KK. 2003. Antibacterial and antioxidant activities of grape (Vitis vinifera) seed extracts. Food Res Int 36:117-22.

Levy RJ, Schoen RJ, Levy JT, Nelson AC, Howard SL, Oshry LJ. 1983. Biologic determinants of dystrophic calcification and osteocalcin deposition in glutaraldehyde-preserved porcine aortic valve leaflets implanted subcutaneously in rats. Am J Pathol 113:143–55.

Mako WJ, Vesely I. 1997. In-vivo and in-vitro models of calcification in porcine aortic valve cusps. J Heart Valve Dis 6:316-23.

Naimark WA, Pereira CA, Tsang K, Lee JM. 1995. HMDC crosslinking of bovine pericardial tissue: a potential role of the solvent environment in the design of bioprosthetic materials. J Mater Sci Mater Med 6:235-41.

Nemes A, Acsady G, Fraefel W, et al. 1985. Application of a vascular graft material (Solcograft-P) in experimental surgery. Biomaterials 6:303-11.

Ogino Y, Osada K, Nakamura S, Ohta Y, Kanda T, Sugano M. 2007. Absorption of dietary cholesterol oxidation products and their downstream metabolic effects are reduced by dietary apple polyphenols. Lipids 42:151-61.

Packer L, Rimbach G, Virgili F. 1999. Antioxidant activity and biologic properties of a procyanidin-rich extract from pine (Pinus Maritima) bark, pycnogenol. Free Radic Biol Med 27:704-24.

Park KD, Yun JY, Han DK, et al. 1994. Chemical modification of implantable biologic tissue for anti-calcification. ASAIO J 40:M377-82.

Pereira CA, Lee JM, Haberer SA. 1990. Effect of alternative crosslinking methods on the low strain rate viscoelastic properties of bovine pericardial bioprosthetic material. J Biomed Mater Res 24:345-61.

Pettenazzo E, Deiwick M, Thiene G, et al. 2001. Dynamic in vitro calcification of bioprosthetic porcine valves: evidence of apatite crystallization. J Thorac Cardiovasc Surg 121:500-9.

Plumb GW, De Pascual-Teresa S, Santos-Buelga C, Cheynier V, Williamson G. 1998. Antioxidant properties of catechins and proanthocyanidins: effect of polymerisation, galloylation and glycosylation. Free Radic Res 29:351-8.

Rapoport HS, Connolly JM, Fulmer J, et al. 2007. Mechanisms of the in vivo inhibition of calcification of bioprosthetic porcine aortic valve cusps and aortic wall with triglycidylamine/mercapto bisphosphonate. Biomaterials 28:690-9.

Schinke T, McKee MD, Karsenty G. 1999. Extracellular matrix calcification: where is the action? Nat Genet 21:150-1.

Schmitz-Rixen T, Megerman J, Anderson JM, et al. 1991. Longterm study of a compliant biological vascular graft. Eur J Vasc Surg 5:149-58.

Schoen FJ. 1999. Future directions in tissue heart valves: impact of recent insights from biology and pathology. J Heart Valve Dis 8:350-8.

Schoen FJ, Levy RJ. 1999. Tissue heart valves: current challenges and future research perspectives. J Biomed Mater Res 47:439-65.

Schoen FJ, Levy RJ. 2005. Calcification of tissue heart valve substitutes: progress toward understanding and prevention. Ann Thorac Surg 79:1072-80.

Schoen FJ, Levy RJ, Nelson AC, Bernhard WF, Nashef A, Hawley M. 1985. Onset and progression of experimental bioprosthetic heart valve calcification. Lab Invest 52:523-32.

Schoen FJ, Tsao JW, Levy RJ. 1986. Calcification of bovine pericardium used in cardiac valve bioprostheses. Implications for mechanisms of bioprosthetic tissue mineralization. Am J Pathol 123:134-45.

Schmidt CE, Baier JM. 2000. Acellular vascular tissues: natural biomaterials for tissue repair and tissue engineering. Biomaterials 21:2215-31.

Shahat AA, Cos P, De Bruyne T, et al. 2002. Antiviral and antioxidant activity of flavonoids and proanthocyanidins from Crataegus sinaica. Planta Med 68:539-41.

Simionescu DT. 2004. Prevention of calcification in bioprosthetic heart valves: challenges and perspectives. Expert Opin Biol Ther 4:1971-85.

Simon P, Kasimir MT, Seebacher G. 2003. Early failure of the tissue engineered porcine heart valve SYNERGRAFT in pediatric patients. Eur J Cardiothorac Surg 23:1002-6.

Sugiyama H, Akazome Y, Shoji T, et al. 2007. Oligomeric procyanidins in apple polyphenol are main active components for inhibition of pancreatic lipase and triglyceride absorption. J Agric Food Chem 55:4604-9.

Valente M, Bortolotti U, Thiene G. 1985. Ultrastructural substrates of dystrophic calcification in porcine bioprosthetic valve failure. Am J Pathol 119:12-21.

Wilson GJ, Courtman DW, Klement P, Lee JM, Yeger H. 1995. Acellular matrix: a biomaterials approach for coronary artery and heart valve replacement. Ann Thorac Surg 60(2 Suppl):S353-8.

Zhai W, Chang J, Lin K, Wang J, Zhao Q, Sun X. 2006. Crosslinking of decellularized porcine heart valve matrix by procyanidins. Biomaterials 27:3684-90.

Zhao J, Wang J, Chen Y, Agarwal R. 1999. Anti-tumor-promoting activity of a polyphenolic fraction isolated from grape seeds in the mouse skin two-stage initiation-promotion protocol and identification of procyanidin B5-3′-gallate as the most effective antioxidant constituent. Carcinogenesis 20:1737-45.

Zilla P, Brink J, Human P, Bezuidenhout D. 2008. Prosthetic heart valves: catering for the few. Biomaterials 29:385-406.