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Journal
The Annals of Thoracic Surgery, 97(4)

ISSN
0003-4975

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Publication Date
2014-04-01

DOI
10.1016/j.athoracsur.2013.11.009

Peer reviewed

The Effects of Transcatheter Valve Crimping on Pericardial Leaflets

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Background. **Transcatheter aortic valve replacement has emerged as a promising therapy for treatment of severe aortic stenosis. Although it has been shown that these valves can be safely delivered and implanted, studies of valve longevity are lacking because of the infancy of the technology. Particularly, the effects of stent crimping on the valve's leaflets have not yet been sufficiently investigated. In this study, we have characterized the effects of crimping on pericardial leaflets in time and through the depth of the tissue.**

Methods. **To test the structural changes at the surface and deep layers of bovine pericardial leaflets, scanning electron microscopy and second-harmonic generation microscopy were used. An uncrimped tissue sample was imaged, followed by imaging a segment of tissue after crimping in a stented transcatheter valve, immediately after, at 20 minutes, and 60 minutes after crimping. The crimping experiment was performed for multiple crimping sizes (ie, 14F, 16F, and 18F). We defined a damage index that quantifies the level of leaflet structural changes as a result of crimping.**

Results. **Based on the calculated damage indices and analyses of the raw images, it was determined that crimping does measurable damage to the leaflet tissue that persists with time.**

Conclusions. **Significant tissue damage was observed at the surface layers of the leaflets. In the deeper tissue layers, damage was substantial for 14F crimping; however, it became less significant but still visible for larger collapse profiles. Crimping may induce substantial structural damage to pericardial leaflets that does not improve with time.**

(Ann Thorac Surg 2014;97:1260–6)

Currently, transcatheter aortic valves are being widely implanted in Europe and have recently gained approval in the United States [1]. With substantial evidence that patients who are categorized as high risk for traditional surgery can now undergo a valve replacement that provides them with a mortality benefit over medical therapy, this technology is here to stay and will only see expanded indications in the future [2].

Percutaneous valves share some characteristics with bioprosthetic surgical valves, but also have many unique properties [3]. The most important similarity is the use of pericardial tissue leaflets, whereas their major difference is the housing of their valves' leaflets within a stent, and the exposure to crimping before implantation [4]. The crimping process is currently performed immediately before implantation and leaves the pericardial leaflets crimped within a compressed stent before delivery [4]. By doing this, the valves' leaflets are exposed to a level of stress that is not exerted on the surgical bioprosthetic valves [5]. There is currently little quantitative data that assess the damage, if any, that results from crimping [6–8]. It is well documented that mechanical stresses applied to pericardial leaflets result in disruption of the collagen fibers' natural pattern, and leads to calcification and early valve failure [6, 9]. Whether this damage is transient or permanent is also a critical question, along with consideration of what effects this may have on the durability of transcatheter valves compared with surgical bioprosthetic valves [10].

In this study, pericardial tissue leaflets were exposed to different levels of stent crimping to sizes of 14F, 16F, and 18F, and were studied under scanning electron and second-harmonic generation microscopes as a function of time to determine the extent and nature of the potential damage caused by crimping based on a novel damage index.

Material and Methods

Sample Preparation

Glutaraldehyde-fixed bovine pericardial tissue sheets (Neovasc, Richmond, BC, Canada) that were cross-linked with 0.50% 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES) -buffered glutaraldehyde (pH 7.4) for storage at 2° to 8° C were used. To image the intact tissue, two small segments from each sheet (n = 10) with uniform thickness of 0.5 mm were cut to be used for second-harmonic generation (SHG) and scanning electron microscopies (SEM). The remaining segments of each sheet were cut into the shape of leaflets and sewn to a nitinol stent by an experienced technician to form a trileaflet heart valve (Fig 1A). Stents used were clinical quality self-expandable

Accepted for publication Nov 8, 2013.

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Published by Elsevier Inc

0003-4975/$36.00
http://dx.doi.org/10.1016/j.athoracsur.2013.11.009

nitinol stents cut from a 4-mm tube (12F) that is expandable to 25 mm. The stent crimping was performed at different sizes of 14F, 16F, and 18F by using a stent crimper for 20 minutes (Fig 1B). This duration as the average time that it takes for crimping and advancement before deployment of the valve at the aortic position was selected after checking with Prof Friedrich Mohr (personal communication). The tissue segments were kept hydrated during microscopy by using phosphate-buffered saline solution (Gibco, Carlsbad, CA).

Scanning Electron Microscopy

Surface structural changes of crimped and uncrimped tissue segments were compared using SEM. The tissue segments were rinsed twice in double-distilled water. They were then dehydrated using ethanol and hexamethyldisilazane (Ted Pella, Inc, Redding, CA) before mounting them on carbon stubs and sputter coating. A low-voltage, high-resolution SEM (Quanta 3D FEG electron microscope, FEI, Hillsboro, OR) was used at 10 kV to investigate the changes of surface morphology for crimped and intact tissue segments.

Second-Harmonic Generation Microscopy

Structural changes of crimped and intact samples within the tissue were compared using SHG microscopy. We acquired SHG images through the depth of tissue using a Zeiss LSM Meta 510 laser microscope (Carl-Zeiss-Strasse, Oberkochen, Germany). The microscope was equipped with a Ti:Sapphire (Chameleon-Ultra, Coherent, Inc, Santa Clara, CA) femtosecond laser source tunable from 690 to 1,040 nm. To image the collagenous structure, the SHG microscope was excited with 900 nm light and filtered from 450 nm to 465 nm. Field-of-view for each image covered 450 μm × 450 μm of each tissue segment (Fig 1C). First, the intact tissue was imaged, and then the crimped tissue was imaged immediately after crimping, then 20 minutes, and finally 1 hour after crimping. The imaging was repeated on three different locations for each leaflet segment.

Damage Index

The acquired SHG images were analyzed by using an in-house developed MATLAB (Mathworks, Natick, MA) code. A damage index (DI) was defined that quantifies the level of leaflet structural changes caused by crimping. The index is described as the area of the largest crack in each image normalized to the area of the whole image. The natural gaps between the fibers in intact states were not considered as damage so those numbers were deducted from crimped DIs.

Statistical Analysis

An unpaired Student's *t* test was performed by using the R software package for Windows (Institute for Statistics and Mathematics, Vienna University, Vienna, Austria). All indices extracted from SHG images were reported as mean ± standard deviation. Probability values less than 0.05 were considered statistically significant.

Results

Contact Surface

Changes caused by crimping on the contact surface were visualized using SEM. Figures 2A, 2C, and 2E show the SEM images taken from the contact surface of three different uncrimped tissue segments at the same magnification. Figures 2B, 2D, and 2F show the contact surface of the crimped leaflets at a similar magnification for 18F, 16F, and 14F, respectively. The leaflets crimped within the stent show a wrinkling surface with several cracks at the micrometer scale. The cracks become larger with increased crimping, and it appears that the surface is significantly pleated at 14F crimping (Fig 2F). Comparison between the surface of uncrimped and crimped pericardial leaflets suggests that crimping results in superficial structural change, and this structural damage is enhanced by increased crimping from 18F to 14F (Fig 2).

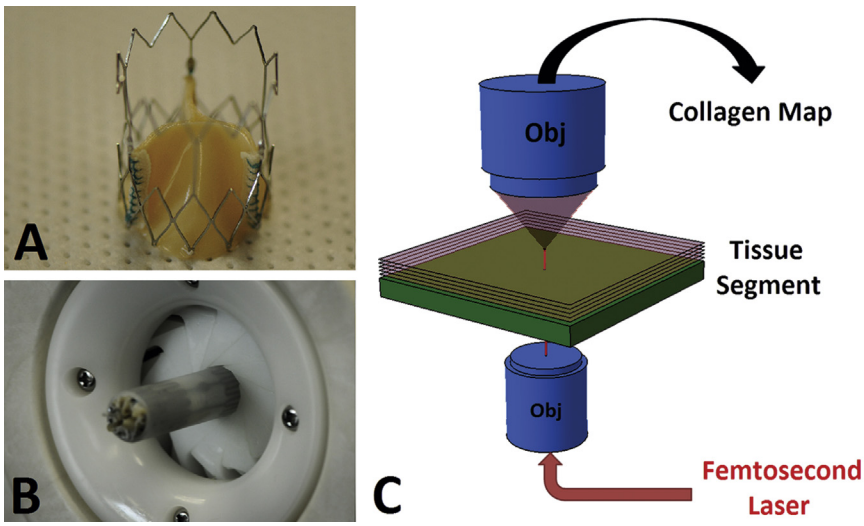


Fig 1. (A) A 25-mm stented valve. (B) Stented valve was crimped to multiple sizes by using a standard stent crimper for 20 minutes before imaging. (C) Schematic representation of second-harmonic generation microscopy on pericardial leaflets.

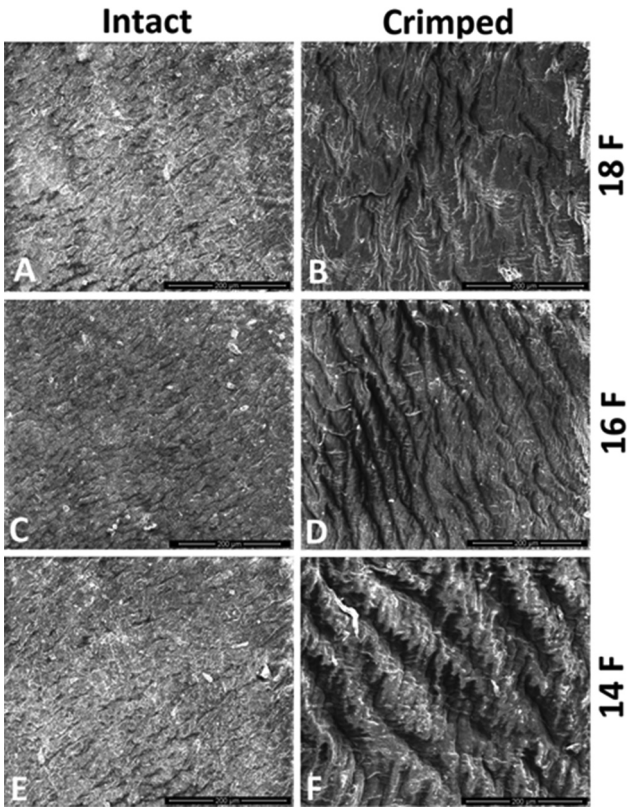


Fig 2. Scanning electron microscopy images show intact (A, C, and E) and crimped (B, D, and F) states of three different pericardial leaflets at 18F, 16F, and 14F, respectively. Comparison of scanning electron microscopy images demonstrates substantial changes on the surface microstructure owing to crimping, which increased with reduction of the collapsed profile.

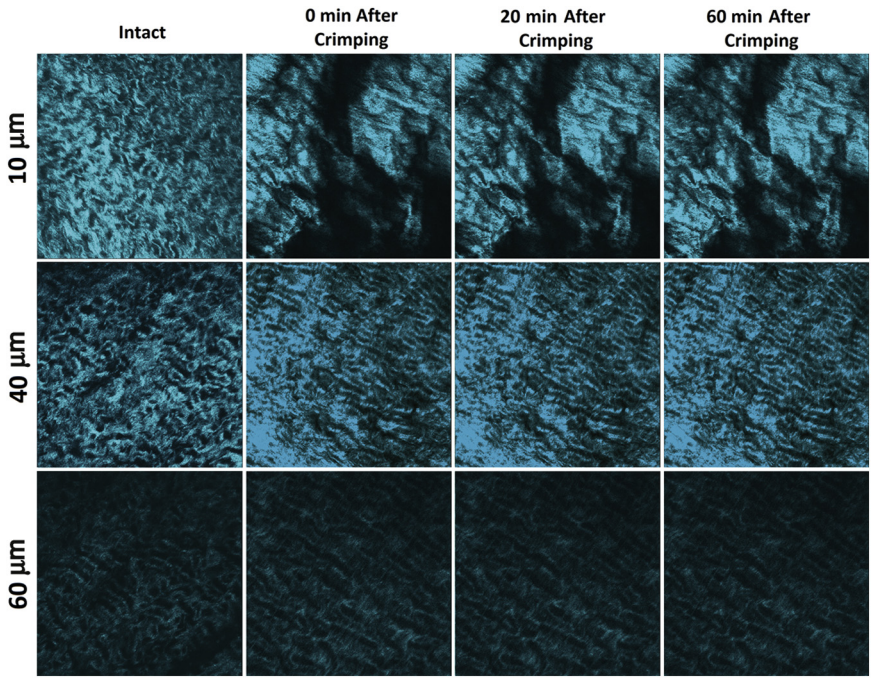
Deeper Layers

We used SHG to map the collagen fiber arrangement deeper into the thickness of the leaflets. Image stacks were collected for each segment of bovine pericardial tissue for both uncrimped and crimped states. Each segment was imaged at depths varying from 10 to 60 μm from the tissue surface. For crimped tissues, the images were acquired right after crimping and at 20 minutes and 60 minutes after crimping for the same location on the tissue to see whether crimping has any permanent effects. Additionally, the crimping was performed at different levels to compare the effects of increased crimping on leaflet structure. To extract and analyze the small differences among SHG images, the DI for each image was calculated as described earlier.

Figure 3 shows the collagen map in both uncrimped and crimped segments in time and depth for samples crimped to a size of 18F. As can be observed, the stent-crimped tissue shows multiple collagen defects at the depth of 10 μm (superficial layers), which did not improve with time. The DI did not change 60 minutes after crimping ($p = 0.9476$). At a 40-μm depth from the leaflet surface, the collagenous structure (ie, fibers and their arrangement) of the tissue changed considerably; however, the rate of the damage was found to be significantly less (approximately 3% DI right after crimping). At a 60-μm depth, the damage was relatively insignificant (approximately 2% DI), and the collagen fibers retain their normal structure. Damage did not improve with time at all depths.

Figure 4 shows the collagen map in both uncrimped and crimped segments for 16F with respect to the time and depth of imaging. The nature of the damage in

Fig 3. Second-harmonic generation microscopy images compare the structural changes of intact and crimped pericardial tissues at the depth of 10, 40, and 60 μm for crimping size of 18F as a function of time.



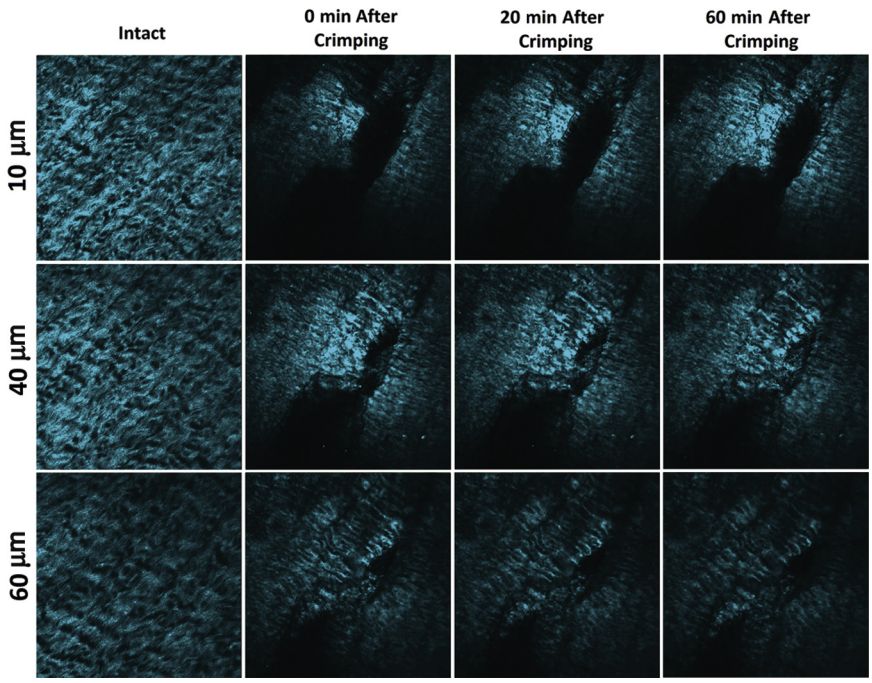


Fig 4. Second-harmonic generation microscopy images compare the structural changes of intact and crimped pericardial tissues at the depth of 10, 40, and 60 µm for crimping size of 16F as a function of time.

superficial layers of 16F crimping was visually very similar to that of 18F; however, within the depth of the leaflet (deeper than 20 µm), different responses were observed. Although it was still significantly more than with 18F crimping, the damage was less in deeper layers with respect to the superficial layers. The collagen fibers did not retain their normal characteristics even at the depth of 60 µm.

Finally, 14F-crimped leaflets showed dramatic structural changes with multiple cracks at all depths (Fig 5). A significant difference between the DIs of 14F crimping compared with those of 16F crimping ($p = 0.0011$) and 18F

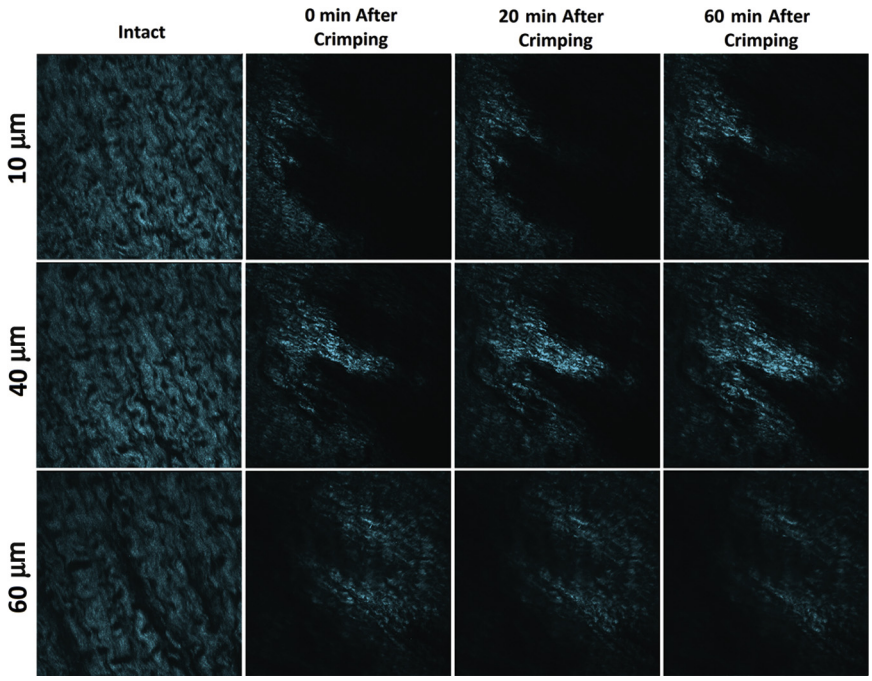


Fig 5. Second-harmonic generation microscopy images compare the structural changes of intact and crimped pericardial tissues at the depth of 10, 40, and 60 µm for crimping size of 14F as a function of time.

crimping ($p = 0.0002$) were observed immediately after crimping. The DIs for 16F crimping and 18F crimping were statistically similar ($p = 0.1188$). With time, the differences followed the same pattern. Data related to the DI were in agreement with the SHG images showing the rate of damage is significantly higher for 14F crimping compared with 16F crimping and 18F crimping for all times and depths.

Figures 6A to 6C show the DI deduced from SHG images with respect to time and depth of imaging at 18F, 16F, and 14F crimping, respectively. As can be seen in Figure 6A, for 18F-crimped leaflets, significant differences ($p = 0.04058$) were observed between the superficial layers (<20 μm) and the deeper layers (≥20 μm). There was no significant ($p = 0.3155$) difference between the DIs at the deeper layers. Additionally, the changes in DI with time were statistically insignificant ($p = 0.9977$). For 16F-crimped leaflets, the rate of reduction of DI with respect to depth was almost linear (Fig 6B). There was no significant difference between the DIs of the layers with a 10-μm depth interval. Additionally, the response in time was similar for all the layers. For 14F-crimped leaflets, the DI was extremely high (approximately 73% at 10 μm to approximately 49% at 60 μm) in all the layers because more than 50% of the area of the leaflets was affected (Fig 6C). The structural changes were sustained in deeper layers with a constant rate of damage in layers deeper than 30 μm. Again, the response of the leaflets as a function of time was found to be negligible at all layers.

The responses as a function of time for all the samples illustrate that the structural changes were not improved during the 1-hour rest period the tissue was given after crimping. As a result of this finding, it is likely that there will be permanent damage to the leaflet tissue after crimping. This permanent damage penetrates through the depth of the tissue with a reduction of the stent collapse profile.

Comment

Transcatheter valve therapies have been a tremendous breakthrough in the treatment of valvular heart disease, particularly in those whose surgical risk precludes them from surgical valve replacement [1]. Currently in the United States a clinical trial (US Clinical Trial NCT01314313) has recently been completed with intermediate-risk patients, and it is not a far stretch of the imagination to assume the indications will further expand with time. Thus an important question arises as to the lifespan of these valves [11]. Although manufacturers have subjected their valves to numerous in vitro tests, there is no substitute for human data, and thus the proverbial jury is still out on longevity, particularly given the fact that the first human case description was only published in 2002, and US Food and Drug Administration approval was only recently granted for a transcatheter aortic valve [12].

Follow-up data for human implants are only available for the last 5 years, with no concrete evidence of early failure [13]. These data, however, are limited by the infancy of the technology, and thus whether a transcatheter

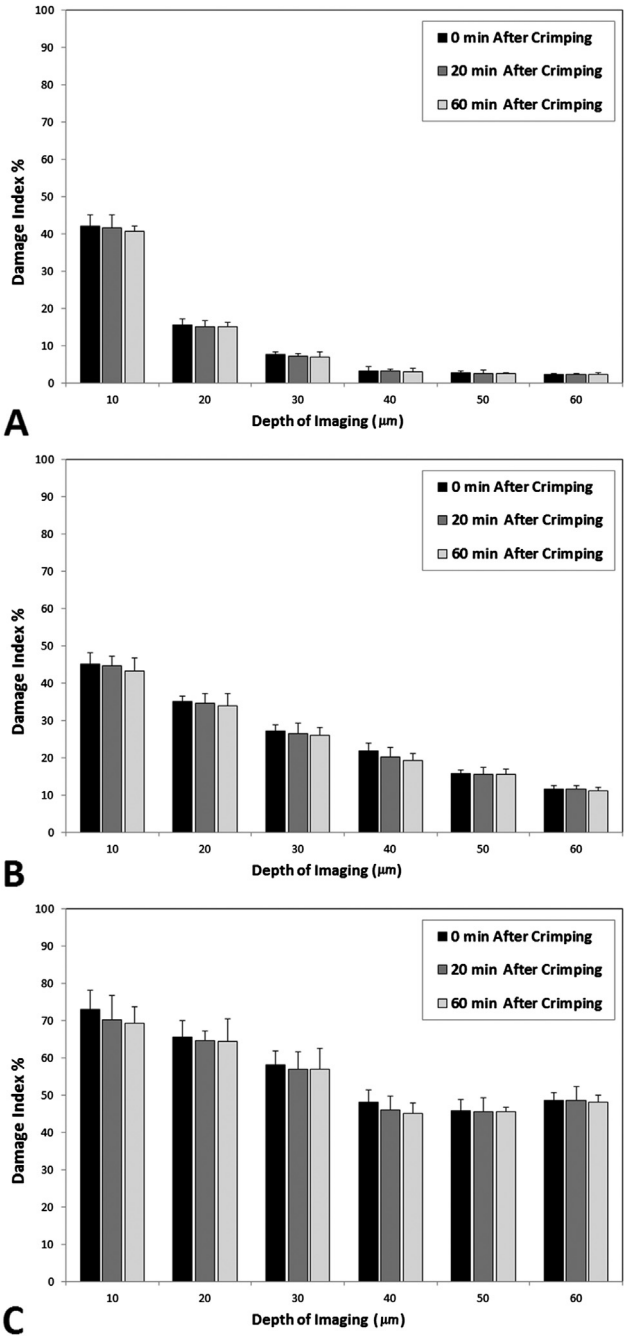


Fig 6. Collected damage indices (DIs) for crimped pericardial tissues with respect to time and the depth of imaging. Second-harmonic generation microscopy imaging was performed with intervals of 10 μm to a maximum 60-μm depth from the tissue surface. Imaging was done immediately after crimping and at 20 minutes and 60 minutes after crimping for each tissue segment. Insignificant changes are observed as a function of time in all groups, meaning damage is permanent. (A) 18F: significant differences are observed between the superficial layers and deeper layers. (B) 16F: linear reduction in damage indices is observed between the superficial layers and deeper layers. (C) 14F: enormous structural changes are observed for both the superficial layers and deeper layers. Damage indices are almost constant after 30 μm.

valve is as durable as a surgical bioprosthetic valve remains to be seen. Additionally, the studied valves are either first or second generation, which were generally crimped to 20F or larger. The results of this analysis are pertinent to the current and future generations that aim to possess smaller delivery profiles.

Valve longevity in patients with high surgical risk is less of a concern, as these patients have a high 5-year mortality rate; thus there is a greater likelihood of death before any structural valve failure. However, in patients who are at low-to-intermediate risk for cardiothoracic surgery, there is a more pressing need to determine whether a transcatheter valve has comparable durability to a surgical bioprosthetic valve [6]. In the PARTNER Trial, patients were required to have a Society of Thoracic Surgeons risk score of at least 10%, putting these patients in a high-risk group [2]. The success of this trial would suggest that transcatheter aortic valve replacement may see expanded indications in lower risk patients. With reduced surgical and long-term mortality, leaflet durability will be of increased concern and a critical component of the viability of transcatheter aortic valve replacement as a substitute for surgical aortic valve replacement.

The major difference between a transcatheter valve and a surgical valve before implantation is crimping. In the operating suite, the valve is prepared, crimped, and placed into the delivery system [4]. The valve may remain crimped for some time, depending on the nature and complexity of the procedure [4]. Given the fixative used in commercial valves, the valve must remain immersed in a liquid, thus not allowing for preloaded delivery systems [14]. However, there are processes in development for fixation that will no longer require aqueous conditions to maintain leaflet integrity, and thus future transcatheter aortic valve replacement systems may come with a preloaded valve [14]. Because of the nature of the preparation process and the potential for increased crimping time, it was important to evaluate the detrimental effects this process would have on the tissue leaflets [15]. For instance, de Buhr and colleagues [7] studied the effect of balloon expansion on leaflet structure. They observed disruption of collagen fibers in pericardial tissues, confirmed by purely qualitative histology [16]. The use of more-advanced imaging techniques allows for better quantification of the damage induced by crimping in three dimensions and with time [6].

Through the experimental process described earlier, it has been determined that stent crimping does indeed produce measurable changes in tissue leaflets. We have also shown that these changes can be quantified and are statistically significant. Additionally, structural changes penetrate through the depth of the tissue with a reduction in stent profile. Not only does this change occur immediately but it persists and is likely permanent. Whether this irreversible damage has any effect on leaflet performance and longevity is not yet known, and will be determined with time and patient experience.

The predominant load components exerted on the leaflets are radial stresses during crimping, which damages the microstructure of the valve along with compression within a structure with a significantly higher stiffness (ie, metallic stent). Given the significant difference between the modulus of elasticity of a leaflet and a stent, regardless of the type of metal, using a different stent material would not have any significant effect on tissue damage. Bovine pericardial tissue was used, which shares a similar collagen pattern to porcine tissue. However, bovine pericardium is richer in hydroxyproline (collagen) content, making it stronger than porcine pericardium. Thus we anticipate the extent of damage would be even more prominent if porcine pericardial tissue were used.

Materials science has taught us that anytime a structure such as a heart valve leaflet is altered and exposed to repeated high stresses, it will experience increased fatigue that may lead to premature failure [17]. Certainly the significant damage to the collagen network induced by crimping will make the tissue less durable and may impair its ability to dampen loads and withstand the constant stresses a heart valve is exposed to [18]. Other important considerations are possible increase in the incidence of prosthetic valve endocarditis and valve thrombosis with transcatheter valves. Microdisturbances in right-side heart valves owing to particulate matter lead to an increase in right-side endocarditis in drug abusers; might the disruptions caused by crimping increase the risk for prosthetic valve endocarditis in those who have undergone transcatheter valve implantation [19]? Or might these crimping-induced defects result in valve thrombosis, as it has been previously proposed that early valve thrombosis can result from leaflet disruption [7]? Finally, valve function may be impaired as glutaraldehyde-fixed xenograft tissue has a great deal of elasticity and pliability, and the damage induced by crimping may take away some of this, impairing leaflet function.

In this study, the tissue leaflets were exposed to crimping for 20 minutes. This is a conservative estimate of the time that a transcatheter valve's leaflets would be in a crimped state [5, 20]. Thus, the fact that there was significant damage in the deeper tissue layers, up to 60 μm, at 14F crimping is concerning. Although this damage was not as substantial as the surface damage for all crimping sizes, it may be a predictor of further damage that would occur once the valve is crimped for a longer period. Although surface damage may cause potential complications such as hemolysis or endocarditis as discussed earlier, deeper structural damage may lead to mechanical failure of the leaflet.

The results of this study suggest that if the leaflets are aggressively crimped, they are severely damaged. Therefore, either the leaflets should be outside the stent during crimping or they should not be aggressively compacted. These data may also indicate that the drive for lower-profile devices should be tempered and perhaps the more surgical-driven apical approach or femoral cutdown is not something that should be abandoned as larger French size catheters are used.

A limitation of our study was the fact that we crimped the tissue valve samples for only 20 minutes. Although this did

provide the important piece of information that any crimping, even for a short time, induces irreversible damage to the leaflets, it does not allow for testing of the possibility that even further destruction of the leaflets is caused by prolonged crimping. Future investigations should also focus on the effects of recrimping to reposition the valve, as this is currently used by some of the novel technologies, which are currently under investigations.

Mechanical testing of the leaflets after crimping should also help determine whether the disruptions seen through SEM and SHG do indeed result in compromising the physical strength of the leaflet. This was beyond the scope of this study, but worth further pursuit. Finally, we were unable to use a commercially available transcatheter aortic valve replacement system owing to legal roadblocks.

By examining the effects that crimping has on tissue leaflets, we have illustrated an important consideration when choosing a therapeutic option for a patient and provided a mechanistic explanation for possible early failure and other complications in patients treated with transcatheter valves. We have shown for the first time that crimping may cause structural damage to pericardial tissue leaflets that persists with time.

When deciding whether to use this emerging technology for treating a patient, much care must be paid to the projected longevity of the patient, as transcatheter valves may not be as durable as surgical bioprosthetic valves owing to the described effects of crimping. Future valve design may be optimized by excluding or minimizing the exposure of the leaflets from crimping. Although the result of the study is not directly related to any of the commercially available heart valves, it will provide sufficient quantitative information about crimp-induced damage to pericardial leaflets. Time will ultimately prove whether these structural damages adversely affect the function of these valves in vivo. However, physicians should be aware of crimp-induced damage to the leaflet whether or not they adversely influence the quality or durability of the valves.

This work was supported by a Coulter Translational Research Award (CTRA) from the Wallace H. Coulter Foundation.

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