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ORIGINAL ARTICLE

Crimping and deployment of balloon-expandable valved stents are responsible for the increase in the hydraulic conductance of leaflets

Channing Convelbo[a], Pierre Guetat[a], Michèle Cambillau[b], Bachir Allam[c], Patrick Bruneval[d], Antoine Lafont[a,c,e] and Rachid Zegdi[a,e,f,*]

[a] Inserm U970, Paris V, Paris, France
[b] Department of Biochemistry, AP-HP, Hôpital Européen Georges Pompidou, Paris, France
[c] Department of Cardiology, AP-HP, Hôpital Européen Georges Pompidou, Paris, France
[d] Department of Pathology, AP-HP, Hôpital Européen Georges Pompidou, Paris, France
[e] René Descartes University, Paris V, Paris, France
[f] Department of Cardiovascular Surgery, AP-HP, Hôpital Européen Georges Pompidou, Paris, France

* Corresponding author. Service de Chirurgie Cardiovasculaire, Hôpital Européen Georges Pompidou, 20 rue Leblanc, 75908 Paris, France. Tel: +33-1-56093748; fax: +33-1-56092219; e-mail: rzegdi@hotmail.com (R. Zegdi).

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Abstract

OBJECTIVES: Leaflet injury has been documented to occur during the deployment of valved stents (VSs). The pathological aspects, however, of this injury are difficult to quantify. Conversely, the hydraulic conductance of a (pericardial) membrane may be easily determined. The impact of crimping and deployment of VS on this parameter was therefore investigated.

METHODS: Bovine pericardial square (25 × 25 mm) patches were placed within a pressure chamber and their hydraulic conductance was determined. The influence of the pressure gradient and tissue thickness on this parameter was analysed. Six balloon-expandable VS were constructed. The hydraulic conductance of their bovine pericardial leaflets was determined before and after VS crimping and deployment in four of them. Pericardial leaflets of non-crimped VS were used as controls.

RESULTS: Hydraulic conductance increased insignificantly with the pressure level within the chamber: from 128 ± 26.9 ml/h/m^2/mmHg at a pressure of 50 mmHg to 232.3 ± 51.9 ml/h/m^2/mmHg at a pressure of 250 mmHg ($P = 0.117$). Hydraulic conductance was not correlated to pericardial thickness, for thickness measurements ranging from 0.34 to 0.76 mm. The hydraulic conductance of VS leaflets significantly increased immediately after crimping from 45.2 ± 7.6 to 667.9.0 ± 527.2 ml/h/m^2/mmHg ($P < 0.001$). This increase was still observed 24 h after VS deployment. No change in hydraulic conductance occurred in the control group.

CONCLUSIONS: The determination of the hydraulic conductance of pericardial patches was easy to perform, reproducible and not influenced by tissue thickness. The hydraulic conductance of pericardial leaflets dramatically increased after VS crimping and deployment. This parameter might be, in the future, a useful noninvasive tool in studying leaflet trauma.

Keywords: Pericardium • Leaflets • Percutaneous valves • Injury • Hydraulic conductance

INTRODUCTION

Valved stent (VS) compression and re-expansion are mandatory steps during transcatheter aortic valve implantation (TAVI). There is growing evidence that these steps are responsible for microscopic leaflet injury. Several groups have observed the presence of tissue compression, fracture or dislocation within leaflets from deployed VS [1–5]. Since long-term (10–15 years) results of TAVI are not available, the impact of these valvular lesions on prosthesis durability remains unknown.

Except in two studies from our group [1, 2], no quantitative data of tissue injury have been provided so far. Lesions are difficult to quantify. A major reason is that their distribution is heterogeneous within the same prosthesis and within the same leaflet. Another is directly related to the nature of the lesions. For example, fractures of collagen bundles may lead to so much debris that is impossible to number. Different cleavages inside tissue thickness may merge and dissociate several times, rendering their numbering difficult. Quantification of lesions is, however, possible, but one should not forget that it is a time-consuming task, and that the final results probably underestimate the true severity of the injury.

To better understand the pathogenesis and the consequences of this tissue trauma, a non-destructive, reproducible and quantitative method of analysis would certainly be useful. From a mechanical point of view, pericardial leaflets may be considered as 'membranes'. Membranes may be characterized by their hydraulic conductance. Some lesions (such as collagen bundles fractures) described in deployed VS may alter this parameter. The purpose of this study, therefore, was first to determine the hydraulic conductance of bovine pericardial membranes and subsequently to evaluate the impact of crimping and deployment of VS on the hydraulic conductance of their pericardial leaflets.

MATERIALS AND METHODS

Materials

Pericardial samples. Fresh bovine pericardium was obtained from a slaughterhouse. Fat was first removed, and samples were stored in a 0.625% glutaraldehyde solution within 6 h after animal sacrifice. There was no other treatment (in particular, no anticalcification treatment).

Samples selected from the anterior part of the pericardium were used for the experiments. Sheets were cut into square (25 × 25 mm) patches. The rough (or fibrous) and smooth (or serous) faces of the pericardial tissue were visually distinguished.

Before each experiment, tissue thickness was measured with a Mitutoyo® micrometer working between 0.01 and 10 mm. The thickness was measured in the centre of each pericardial patch. Values were taken after a stabilization period of 15 s.

Pressure chamber. The pressure chamber (Fig. 1) consisted of a cylindrical tube filled with a saline solution (0.9% NaCl). Pericardial patches were placed between two 'pistons'. The superior and inferior 'pistons' had a central coaxial orifice of 21.5-mm diameter. The chamber was connected by a three-way cock valve to a rotating syringe filled with saline. The three-way cock valve was also connected to a mercurial manometer to measure the pressure within the chamber. A container was placed on a weighting scale positioned under the pressure chamber. The amount of collected fluids was measured and used for the calculation of the hydraulic conductance of the tissue (K_f).

To determine this coefficient, the density of saline was assumed to be $\rho = 1.00$ g/ml. The hydraulic conductance formula was $K_f = m/\rho \cdot t \cdot S \cdot P$, where m (g) corresponded to the mass of the collected fluid within a given time period, t (h) the time period during which the solution was collected, S (m^2) the surface of the pericardium directly in contact with the saline solution and P (mmHg) the pressure within the chamber. All measurements were performed at ambient temperature.

Valved stents construction. Six VS were used in this study. Each VS consisted of three bovine pericardial leaflets mounted onto a metallic stent (Fig. 2). The stent was a 23-mm cobalt–chromium stent used in manufacturing of the balloon-expandable Sapien-Edwards XT bioprosthesis (Edwards Lifesciences, Irvine, CA, USA). Valve leaflets were designed with a square shape (25 × 25 mm). All leaflets had a thickness ranging from 0.40 to 0.60 mm. No tissue skirt was incorporated into the prosthesis.

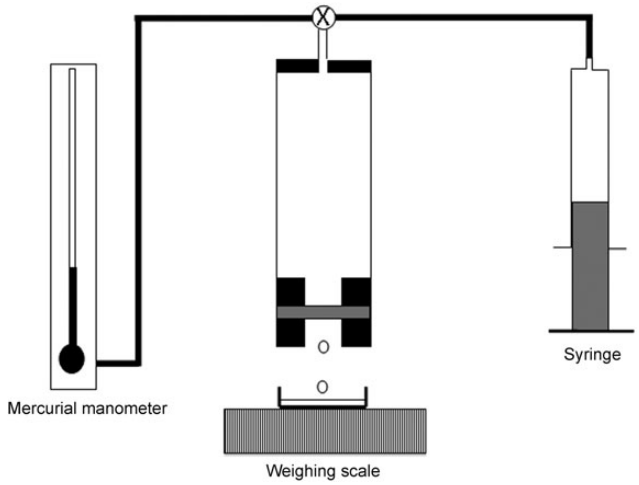

Figure 1: Schematic diagram showing the pressure chamber (filled with saline—middle) used to determine the hydraulic conductance of pericardial sheets. The chamber was connected to an inflation syringe (right) and a manometer (left).

Experimental protocol

Experiment 1. The main goal of this experiment was to evaluate the influence of the duration of exposure to pressure on the K_f value. In this experiment, pericardial patches ($n = 12$) were positioned within the pressure chamber with their smooth surface in direct contact to saline. They were randomly exposed for 28 min to a pressure of either 100 ($n = 6$) or 200 mmHg ($n = 6$). The filtrated volume was recorded every 4 min. Hydraulic conductance of pericardial membranes was determined during the following incremental periods: 4, 8, 12, 16, 20, 24 and 28 min.

Experiment 2. This experiment aimed to evaluate the influence of the pressure level within the chamber on the hydraulic conductance of the pericardial patches. The influence of tissue thickness was also analysed. Pericardial samples ($n = 24$) were therefore exposed to incremental pressure levels. Pressure within the chamber was first set at 50 mmHg and then increased by 50 mmHg every 5 min. The amount of fluids collected before each increase in pressure was used to calculate K_f.

Experiment 3. The purpose of this experiment was to study the impact of VS crimping and deployment on the hydraulic conductance of their leaflets.

The range of variation of the hydraulic conductance of pericardial patches was large (see results from Experiments 1 and 2). In this experiment, pericardial patches with low K_f (≤80 ml/h/m^2/mmHg) were intentionally selected for VS construction.

Six VS were constructed. Four of them were randomly crimped on a Crystal balloon (BALT Extrusion, Montmorency, France) with a specific crimper (Edwards Lifesciences). VS re-expansion was performed by balloon inflation 15 min later (to prevent valve desiccation, crimped prostheses have been immersed in saline). Complete deployment was achieved within 3–4 s. Balloons were maintained fully inflated for 5 additional seconds.

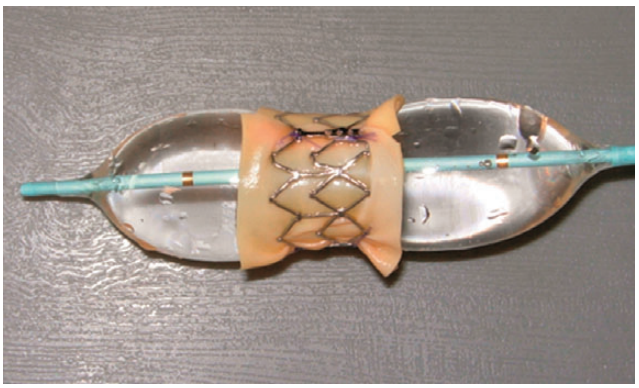

Figure 2: A view of the experimental bovine 'valved stent' during inflation of a Crystal balloon (BALT Extrusion, Montmorency, France).

After balloon deflation, the leaflets ($n = 12$) were carefully removed and immediately tested within the pressure chamber. Hydraulic conductance was determined during a 30-min period during which the pressure level within the chamber was maintained at 150 mmHg. Hydraulic conductance was also determined 24 h later with the same experimental settings (pericardial leaflets were immersed in saline at room temperature between these two experiments).

Two VS were not crimped (control group). Their leaflets ($n = 6$) were carefully removed and subsequently tested the same manner.

At the end of the experiment, all leaflets were processed for histological analysis. After paraffin embedding, pericardial leaflets sections were cut at 5 µm of thickness and stained with Red Sirius. The samples were analysed and digitalized pictures were obtained at ×10.

Statistical analysis

Quantitative variables were expressed as mean ± standard error of the mean. Comparisons between variables were performed using the Kruskall–Wallis or Mann–Whitney's test where appropriate. Correlation between two continuous variables was evaluated with the Spearman's rank-order correlation coefficient. Statistical significance was defined as a P-value <0.05.

RESULTS

Experiment 1

At 100 mmHg, K_f increased from 171.2 ± 78.1 to 202.6 ± 68.8 ml/h/m^2/mmHg at 4 and 28 min, respectively. This increase was not statistically significant ($P = 0.778$). When the collection period was set at 4 min, hydraulic conductance of the pericardial patches remained stable for 28 min ($P = 0.996$; Fig. 2). At 200 mmHg, the period of fluid collection also had no influence on the K_f value of the pericardial patches ($P = 0.998$; Fig. 3A).

Experiment 2

Hydraulic conductance increased from 128 ± 26.9 ml/h/m^2/mmHg at a pressure of 50 mmHg to 232.3 ± 51.9 ml/h/m^2/mmHg at a pressure of 250 mmHg (Fig. 3B). This increase, however, was not statistically significant ($P = 0.117$).

At each level of the pressure chamber (varying from 50 to 250 mmHg), the hydraulic conductance of pericardial patches did not correlate with pericardial thickness (data not shown).

Experiment 3

Baseline K_f was 53.5 ± 7.9 ml/h/m^2/mmHg for the uncrimped control group. This parameter remained stable over a 24-h period ($P = 0.947$; Fig. 4).

In the crimped group, baseline K_f was 45.2 ± 7.6 ml/h/m^2/mmHg. This value did not significantly differ from that of the control group ($P = 0.241$). The hydraulic conductance significantly increased immediately after crimping to 667.9.0 ± 527.2 ml/h/m^2/mmHg ($P < 0.001$; 10 min post-deployment vs baseline). After a 24-h resting period in saline, K_f remained significantly elevated in comparison with baseline: 664.8 ± 410.9 vs 45.2 ± 7.6 ml/h/m^2/mmHg ($P = 0.001$).

Increase in hydraulic conductance notably differed among leaflets. Relative increase in leaflets' hydraulic conductance from baseline to 10 min post-deployment varied from 63.4 to 14 209% (mean = 1412.3%). Even after exclusion of the leaflet with an outlier value, 10-min post-deployment K_f remained significantly higher than baseline: 141 ± 20.2 vs 45.2 ± 8 ml/h/m^2/mmHg ($P < 0.001$). In this case, the mean relative increase in K_f was 248.9% (range: 63.4–491.7%).

Macroscopically, no leaflet tear or dehiscence was visible in any case. At microscopic analysis, the tissue appearance was well preserved in the control group (Fig. 5). Conversely, areas of tissue compression or dislocation and collagen bundles fractures were seen in all crimped pericardial patches (Fig. 5). These lesions had a heterogeneous distribution within the same leaflet. Their severity also differed between leaflets from the same prosthesis.

COMMENT

In the present study, we have shown that hydraulic conductance of the pericardium might be easily determined. This measurement was also reproducible, influenced by the transmembrane pressure but not by tissue thickness. Most importantly, crimping and deployment of balloon-expandable VS were responsible for the traumatic injury and increased hydraulic conductance of the leaflet.

TAVI is a fast-expanding new technology. It is still restricted to ill patients who are considered to be at high risk for surgery [6]. The main reason for this restriction is the absence of long-term data. Prosthesis durability will be a key determinant in the future of this new minimally invasive procedure.

Preservation of the structural integrity of the tissue is thought to be a major determinant of prosthesis durability. From a mechanical point of view, crimping (and expansion with balloon-expandable VS) may be assimilated to a (complex) stress that is applied to the leaflets. Whether leaflet injury occurs depends on the magnitude of this stress on one side and the tissue 'resistance' on the other side. A few studies have shown that the structural integrity of the leaflet was altered during the use of percutaneous valves. Zegdi *et al.* have described several types of lesions inside the pericardial leaflets of deployed Sapien-Edwards bioprosthesis [1]. These lesions mainly consisted of tissue dislocation and collagen bundle fractures. Such lesions have also been described in other reports mainly with home-made balloon-expandable VS [5].

Areas of 'plasmatic insudation' within leaflets of an explanted Sapien-Edwards prosthesis have recently been described [1]. This lesion, also previously described with surgically implanted bioprostheses, suggested the existence of an increased hydraulic conductance (or tissue permeability) of the pericardial leaflets [7]. It appeared to us, therefore, that determination of the hydraulic conductance of the leaflets might be an interesting way to quantify the impact of crimping and deployment of balloon-expandable valves on their leaflets' integrity.

From a mechanical point of view, pericardial leaflets may be considered as 'membranes' and membrane permeability may be easily determined. In the present study, measurement of the hydraulic conductance of pericardial patches was easily done with a pressure chamber. This measurement was found to be reproducible for a given transmembrane pressure gradient with no

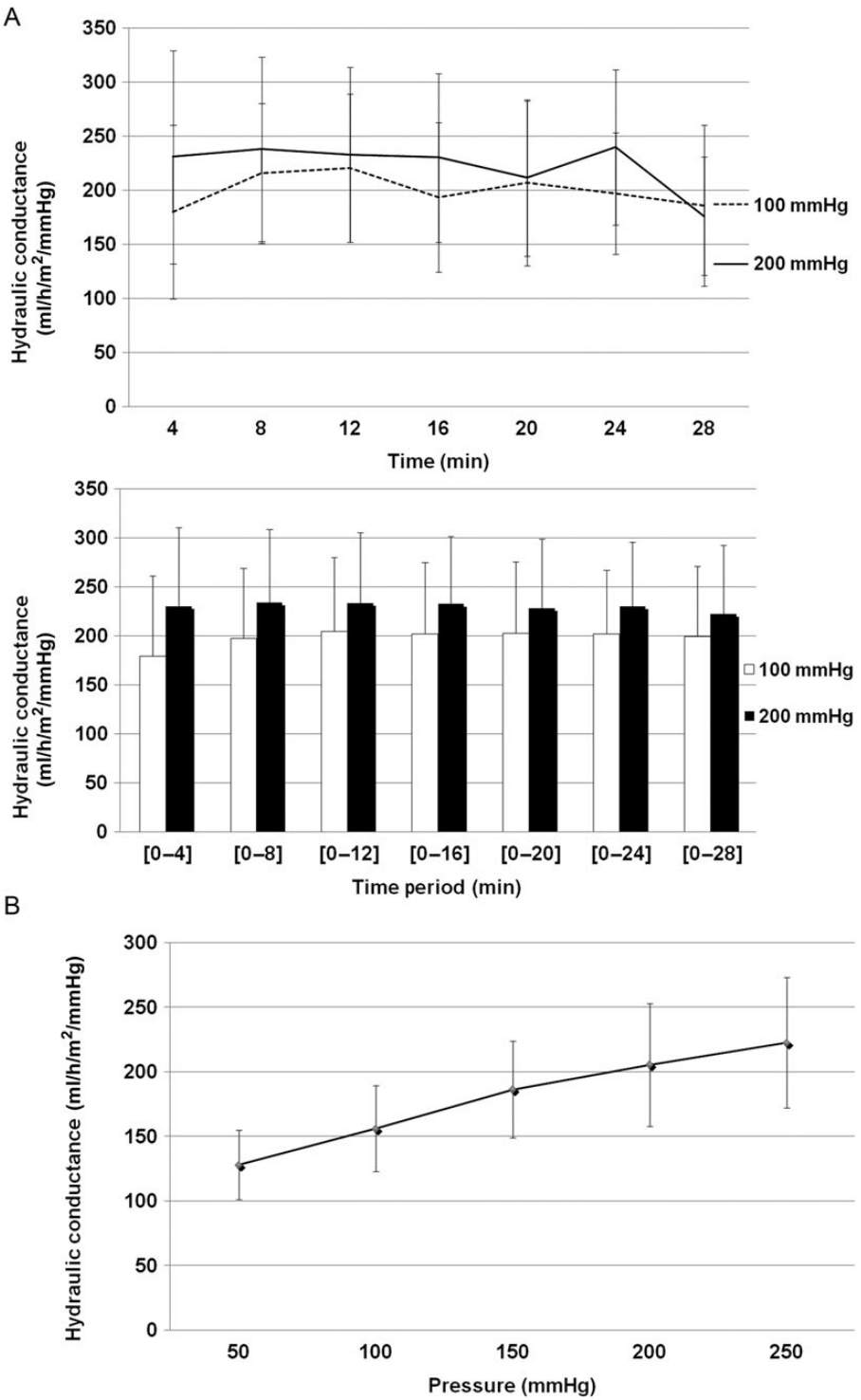

Figure 3: (**A**, Top) Influence of the duration of the period of fluid collection on the hydraulic conductance of pericardial patches. (Bottom) Evolution of the hydraulic conductance (determined during successive 4-min periods) over a 28-min period. (**B**) Influence of the pressure within the chamber on the hydraulic conductance of pericardial patches.

impact of tissue thickness (within the range of 0.34–0.76 mm). The values of hydraulic conductance found were in the same range of that reported by others for canine or human pericardium [8]. Unfortunately, to the best of our knowledge, data for bovine pericardium have not been published so far.

The main finding of the present study was that crimping and deployment of balloon-expandable VS were associated with an average 15-fold increase in the hydraulic conductance of leaflets. The magnitude of this increase varied from one leaflet to another, even within the same VS. This phenomenon was also described with microscopic findings [1, 2]. The reasons for this variability are still unclear. Several hypotheses can be suggested: inhomogeneity in stress distribution during prosthesis crimping and/or expansion; heterogeneity in tissue resistance to stress.

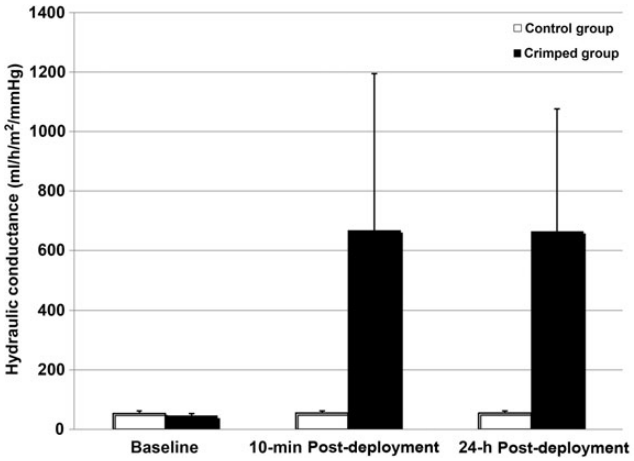

Figure 4: Impact of crimping and deployment of valved stents on the hydraulic conductance of their pericardial leaflets.

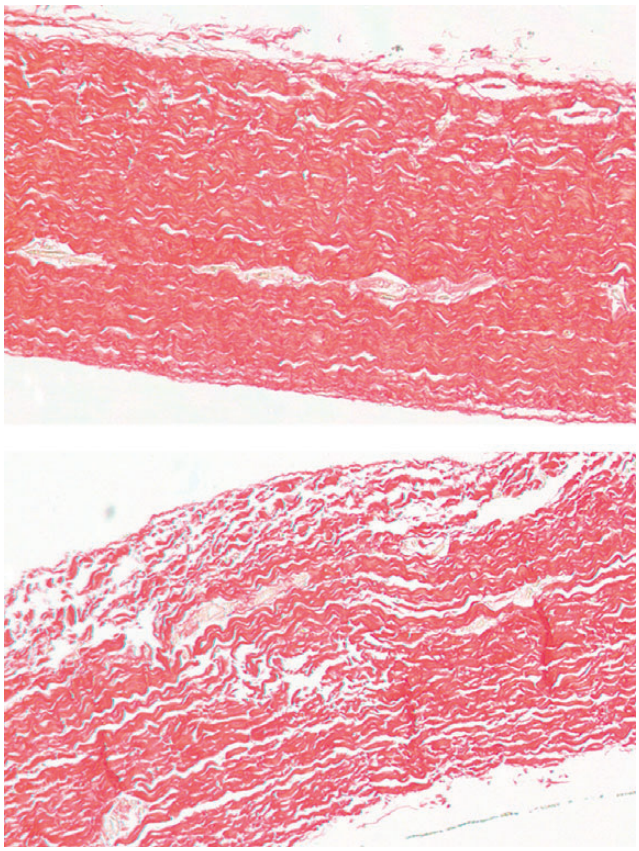

Figure 5: (Top) Microscopic appearance of the control bovine pericardium. The architecture was well preserved. (Bottom) Tissue dislocation and collagen bundle fractures in the bovine pericardium from a balloon-expandable valved stent (Red Sirius staining; ×10 magnification).

The design of the current study, however, did not allow an answer to this question.

Collagen is the main constituent of the pericardium [9]. Structural damage to the collagen network is likely responsible for the increase in leaflet hydraulic conductance of crimped VS as seen in the present study. The relationship between tissue injury (and particularly collagen bundles disruption) and hydraulic conductance of leaflets from deployed VS is the subject of an ongoing study.

Post-traumatic increase in leaflet hydraulic conductance means an increase in leaflet permeability. As a result, an increase in protein and lipid content is expected to occur within the traumatized tissue. This is in accordance with the recent observation of the presence of areas of plasma insudation within leaflets from an explanted Sapien prosthesis [1]. Such lesions have already been described in deteriorated Ionescu Shiley's bioprosthesis and have been associated with areas of tissue calcification [7]. Lipids, furthermore, have been recognized as important tissular binding sites for calcium [10]. Their increased content within injured pericardial leaflets might, therefore, promote calcific degeneration. This aspect of leaflet trauma is also under investigation in our lab.

The selection of pericardium is a critical step in prosthesis manufacturing. A pericardial tissue that is poorly permeable, particularly for lipids and proteins, might be less prone to tissue calcification. In addition, the determination of the hydraulic conductance of the pericardial tissue can be performed on a non-destructive basis. It may also be easily implemented during the valve-manufacturing process. However, its utility and its superiority to the currently used mechanical tests (tissue thickness and tissue elasticity) remain to be established. This method, therefore, cannot be recommended for tissue selection at the present time.

It is difficult to compare the amount of compression between the Sapien prosthesis and our experimental device. On one side, our leaflet thickness (0.4–0.6 mm) was certainly higher than that of the Sapien prosthesis. On the other, the Sapien prosthesis has a tissue skirt, which was not the case with our experimental VS. Whether tissue compression was greater or not in our experiment is, therefore, difficult to establish. We believe, however, that the determination of the hydraulic conductance of leaflets will be a useful tool for the evaluation of the relationship between 'tissue trauma' and the magnitude of crimping.

To conclude, the determination of the hydraulic conductance of pericardial patches was easy, reproducible and influenced by the pressure gradient but not by tissue thickness. The hydraulic conductance of pericardial leaflets dramatically increased after VS crimping and deployment. This parameter might be, in the future, a useful noninvasive tool in studying valve trauma.

Conflict of interest: Rachid Zegdi is a stockowner of the Cormove Company that is developing a new percutaneous valve.

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