A correlation between long-term in vitro dynamic calcification and abnormal flow patterns past bioprosthetic heart valves

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Abstract In this paper, a long-term in vitro dynamic calcification of three porcine aortic heart valves was investigated using a combined approach that involved accelerated wear testing of the valves in the rapid calcification solution, hydrodynamic assessment of the progressive change of effective orifice area (EOA) along with the transaortic pressure gradient, and quantitative visualization of the flow. The motivation for this study was developing a standardized, economical, and feasible in vitro testing methodology for bioprosthetic heart valve calcification, which would address both the hydrodynamic performance of the valves as well as the subsequent changes in the flow field. The results revealed the failure of the test valves at 40 million cycles mark, associated with the critical decrease in the EOA, followed by the increase in the maximum value of viscous shear stress of up to 52%, compared to the values measured at the beginning of the study. The decrease in the EOA was subsequently followed by the rapid increase in the maximum streamwise velocity of the central orifice jet up to the value of about 2.8 m/s, compared to the initial value of 2 m/s, and to the value of 2.2 m/s in the case of a control valve along with progressive narrowing of the velocity profile for two test valves. The significance of the current work is in demonstrating a correlation between calcification of the aortic valve and spatial as well as the temporal development of abnormal flow features.

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1 Introduction

When compared to a mechanical heart valve prostheses, surgical bioprosthetic heart valves are superior in hemodynamic performance and do not require a lifetime anticoagulation therapy. Unfortunately, they may succumb to premature tissue calcification, and, eventually, to a structural valve dysfunction [1–6]. This may significantly decrease the performance and the long-term durability of the surgical bioprosthetic heart valves. The tissue calcification was also observed to affect the performance and durability of the transcatheter aortic valve implants (TAVI) [5, 7–9], which is an emerging and highly effective minimally invasive treatment procedure for severe calcific aortic stenosis in patients at high or prohibitive surgical risk.

Over the last two decades, there have been numerous studies, both *in vitro* and *in vivo*, to determine the mechanism of the premature deterioration of the bioprosthetic heart valve due to tissue calcification and to develop a proper *in vitro* testing methodology that would allow testing a new generation of bioprosthetic heart valves for dynamic calcification [1, 10–15]. Reference [16] is one of the first studies where the calcification solution was developed in order to investigate the capacity of α -elastin in the initial process of calcification in the *in vitro* system. A similar approach was employed by the authors in [10, 11] to study the calcification process in polymeric and bioprosthetic heart valves. Specifically, the authors in both studies demonstrated the capability of an accelerated calcification system to approximate the calcification of heart valve prostheses with physiological concentrations of calcium and phosphate.

A modification to the original *in vitro* accelerated calcification methodology [10, 11] was developed by the group of researchers from Germany and Greece [1, 17, 18]. The main idea behind the developed methodology was to have precise control of the kinetics of the formation of hydroxyapatite, which is the model inorganic compound for the calcified deposits. The authors monitored the initiation of the formation of calcium phosphate deposits at which point a titrant solution, of appropriate concentration aimed to compensate for the precipitated ions, was added in order to reestablish the supersaturation in the working solution [17]. Such innovative modification of the original *in vitro* methodology allowed controlling of the pH and the activities of all ions in the solutions, which resulted in higher calcification rates compared to the previous *in vitro* methodology.

While most of the earlier studies were performed on surgical aortic valves, the logical question arises if the new generation of transcatheter aortic valves (TAV) that is typically used for patients with severe aortic stenosis, is susceptible to a premature calcification. Unfortunately, the loss of the functionality due to premature calcification, a frequently encountered issue in surgical bioprosthetic valves [19, 20], was reported to cause a similar functional failure of TAV [5, 8, 21, 22]. The unique design of some of the TAVs [5, 21] led to leaflet calcification on both the outflow and inflow surfaces, as well as on the valve's frame. Long-term durability evaluation for TAV is essential since many patients that undergo transcatheter valve replacement might not survive a revision surgery if TAV calcification failure occurs [23, 24].

The objective of the current work was to develop a standardized *in vitro* testing methodology for bioprosthetic heart valve calcification, which would address both the hydrodynamic performance of the valves as well as the subsequent changes in the flow field. In particular, the accelerated cycling procedure employed in the present study provides an economical and

feasible way of assessing hydrodynamic performance of heart valves and their susceptibility to calcification that is representative of several years of real-time operation.

A major goal of this study was to determine a correlation between progressive calcification of the aortic valve prostheses and spatial as well as the temporal development of abnormal flow features. In that regard, the study was aimed at providing a better understanding of the fluid dynamics associated with the processes of dynamic calcification of the aortic heart valves as well as developing an effective methodology that could enable efficient determination of the heart valves that are susceptible to premature dynamic calcification, as well as identification of regions within the valve design where the calcification is more dominant.

Two test valves were subjected to calcification medium following the test protocol used in regulatory wear testing, such as ISO 5840–2:2015 [25]. Micro-computed tomography (μ CT) was used to confirm the presence of calcium deposits, while the pre- and post- hydrodynamic functional evaluation and quantitative flow imaging techniques were applied to quantify the degree of calcification and wear. The results obtained for calcified valves were compared to those of the reference valve that was not subjected to the dynamic calcification process.

2 Methods

2.1 Experimental procedure

In this study, three approved porcine surgical aortic heart valve prostheses, with a tissue annulus diameter (TAD) of $D_O = 23$ mm, which are referred further in the text as A, B, and C, were tested to 50 million cycles at a rate of 500 cycles/min in an accelerated wear tester (AWT) system (HiCycle, ViVitro Labs Inc., Canada). The test valves were of the same type, they were produced by the same manufacture, and they were obtained from the same batch, based on their serial numbers. The pressure difference across the valves was set to 100 mmHg. The aortic pressure reading was located 91.7 mm downstream from the valve. The ventricle pressure tracing was located 102.5 mm upstream of the valve. Both readings were taken from the center plane of the valve. Two of the valves, specifically valves A and B, were exposed to an artificial calcification solution [11] described below, which was maintained at 37 °C throughout the duration of the test. The valve C was used as a control valve and was exposed to 0.9% saline also maintained at 37 °C throughout the duration of the test. Testing was conducted in accordance with ISO 5840–2:2015 [25], an international standard that contains standardized methods for evaluating the performance of surgically implanted heart valve substitutes, in particular durability and hydrodynamic testing. As advertised by the manufacturer, all valves were treated with an anticalcification agent containing ethanol and Tween-80, details of which were not disclosed. The calcification solution was monitored for consistent pH of 7.4, and was changed weekly to minimize fluctuation of Ca^{++} and HPO_4^- concentration. Every 10 million cycles, the heart valve prostheses were removed from the AWT and were subjected to hydrodynamic assessment at nominal conditions in accordance with ISO 5840–2:2015 [25]. At the same time, extensive, quantitative flow visualization was performed at the commissure posts of each valve by means of digital particle image velocimetry (PIV). The calcification solution was also refreshed after every iteration of hydrodynamic assessment and quantitative flow visualization.

2.2 Experimental apparatus

In Fig. 1 it is possible to see the schematic of the experimental apparatus that allows evaluation of the heart valves in the aortic configuration during both hydrodynamic testing of the valves and quantitative flow visualization. The flow loop shown in Fig. 1a consisted of a Pulse Duplicator System (ViVITRO Labs Inc., Victoria, Canada), which is a left heart model that is capable of testing and assessing the performance of two heart valve substitutes simultaneously under prescribed cardiac conditions. The flow loop was subjected to a normotensive, physiologically accurate pressure and flow regime: 70 beats per minute, mean aortic pressure was adjusted to 100 mmHg, and the target cardiac output was set to 5 l/min with 35% systolic duration. Blood analog fluid mixture in the ratio of 45:55 (0.9% w.t. saline, 0.5 ml of Cosmocil preservative per 1 l of distilled water, with dynamic viscosity of 3.2 cP, and specific gravity of 1.12 at 37 °C) was used during hydrodynamic and PIV data acquisition. An acrylic, flame-polished, test chamber that represented a simplified model of the ascending section of a human aorta without aortic sinuses, to allow for easier compensation for optical distortions, was

Fig. 1 Experimental system: schematic of the flow loop (a), definition of PIV data acquisition planes (b), and schematic of the test chamber (c)

fabricated. This experimental setup allows visualization of the flow upstream and downstream of the heart valves in the aortic position. The geometry and the dimensions of the acrylic chamber, illustrated in Fig. 1c, were as follows: $W = 50$ mm, $D = 25$ mm, $h = 40$ mm, $H = 100$ mm.

2.3 Calcification solution

Barbital buffer solution [16, 26] was prepared by combining of KCl (99.0%, Sigma), CaCl_2 (99.0%, Sigma), KH_2PO_4 (99.995%, Fluka) and one Barbital buffer tablet (Sigma) to 3 l Nanopure water (18 Mohm cm). Solution preparation was performed in a vessel covered with foil due to the light-sensitive nature of Barbital. The initial pH of the solution (approximately 8.5) was adjusted to a pH of 7.4 with the addition of concentrated HCl. The final concentrations of reagents were equal to 55 mM, 1.5 mM, 1.25 mM, and 20 mM for KCl, CaCl_2 , KH_2PO_4 , and Barbital buffer, respectively. Calcium $\times$ phosphate product, $[\text{ion-Ca} \times \text{Pi}]$, was 1.2 times higher than the normal upper range of $1.57 \text{ mmol}^2/\text{l}^2$ for the healthy individuals [27], although lower than the risk level of $2.24 \text{ mmol}^2/\text{l}^2$.

2.4 Hydrodynamic assessment of the valves

The hydrodynamic assessment of the heart valves was performed using the ViVibro Pulse Duplicator system (ViVibro Labs Inc., Canada) at a beat rate of 70 beats per minute with a systolic duration of 35%, a target cardiac output of 5 LPM, and a mean aortic pressure of 100 mmHg. These conditions represented normotensive conditions as defined in ISO 5840–2:2015 [25]. Simultaneous measurements of the time-dependent flow rate and pressure upstream and downstream of the test valves were performed during ten consecutive cardiac cycles. Clinical identification of the aortic stenosis is typically performed by means of echocardiography or catheter analysis for the effective orifice area (EOA), blood velocity (V), and mean transaortic pressure gradient (Δp) [28–30]. In addition to that, valve tissue mineralization associated with aortic stenosis often lead to some degree of aortic regurgitation, especially in the case of the older patients [31, 32]. In the current study, the same metrics were employed, and the findings were reported based on those major parameters. The effective orifice areas (EOA), mean transaortic pressure gradient Δp , and regurgitation fraction (RF) for the valves were calculated at the first cycle and every 10 million cycles. In particular, the EOA was calculated using the following definition [25]:

$$EOA = Q_{rms} / \left(51.6 \times \sqrt{\Delta p / \rho} \right) \quad (1)$$

where EOA is the effective orifice area in cm^2 , Q_{rms} is the root mean square of the forward flow rate in ml/s , Δp is the mean value of the pressure difference, which was measured over the positive period of the pressure during the forward flow phase in mmHg, and ρ is the density of the test fluid in g/cm^3 . The initial EOA for all three valves was equal to $1.93 \text{ cm}^2 \pm 0.05$. The RF was defined as the volume of fluid that flows through and around a valve in the reverse direction during one cycle and is the sum of the closing and leakage volumes expressed as a percentage of the stroke volume. Such assessment accounted for both transvalvular and paravalvular leakage.

2.5 Micro-computed tomography

The amount of calcium deposits was quantified by using μ CT imaging at the University of British Columbia Centre for High-Throughput Phenogenomics (Vancouver, Canada). All samples were scanned with a resolution of 50 μ m, an energy of 55 kVp, a tube current of 200 μ A, 0.5 mm Al filter and a 0.36° rotation angle on a “ μ CT 100” scanner (Scanco Medical, Brüttisellen, Switzerland). To prevent the samples from drying out while maintaining optimal contrast, they were placed in a plastic tube filled with corn oil [33]. In order to further improve the contrast of the soft tissue, the samples were submerged for 20 min in an iodinated medical CT contrast agent (Iohexol, GE Healthcare, final concentration 17% in water). Data obtained from μ CT imaging was post-processed with the help of the commercial 3D reconstruction environment, Amira v6.0.1 (FEI Visualization Sciences Group, USA). In order to identify calcium deposits by means of μ CT imaging, a custom thresholding procedure that employed a Gaussian statistical distribution with an optimized gray level histogram for the tissue volume, volume of the calcium deposits, and surrounding background was employed to yield an optimal distribution. The obtained distribution was further scrutinized to determine amount of calcium deposits in the sample. The determination of the threshold for calcium deposits was based on comparison with the reference valve.

2.6 Quantitative flow imaging

Quantitative flow imaging was achieved by employing a two-component, two-dimensional DPIV [34, 35]. The PIV system used in the current study utilized a Quantronix Darwin-Duo diode-pumped Nd:YLF dual-cavity laser (maximum energy output of 25 mJ per pulse at a wavelength of 532 nm), which, through a series of cylindrical and spherical lenses, generated a laser sheet with a thickness of 1 mm.

The flow was seeded with fluorescent particles (Fluostar, EBM Corp., Japan) filled with Rhodamine B. The particles had a mean diameter of 15 μ m, 580-nm emission wavelength, and specific gravity of 1.1. A long-pass filter (Midwest LP550–62), with a sharp cut-off wavelength at 550 nm, attached to the camera lens, was employed in order to block background during PIV data acquisition. The images of the tracer particles were recorded using a Complementary Metal Oxide Semiconductor (CMOS) camera (Photron, Japan) equipped with a 1024×1024 pixels sensor with a pixel size of 17 μ m, and which was oriented parallel to the data acquisition plane shown in Fig. 1b. The physical size of the data acquisition plane was equal to 105 mm $\times$ 57 mm, which represented a region extending approximately 1 D_O upstream and 3.6 D_O downstream of the valve. The origin ($X_i = 0$ mm, $Y = 0$ mm) corresponded to the center of the outflow area of the base of the valve, with i – corresponding to the number of the commissure post.

Planar DPIV data was acquired at a rate of 200 image pairs per second. The sequential image pairs were subsequently interrogated using a multiple-pass cross-correlation algorithm with the size of the initial interrogation window equal to 64 pixel $\times$ 64 pixels, and the size of the final interrogation window equal to 16 pixel $\times$ 16 pixels, or 1.64 mm $\times$ 1.64 mm in the physical space. A 50% overlap between the adjacent windows was utilized as well, yielding vector-to-vector spacing equal to 8 pixels, which corresponded to the resolution of 0.8 vector/mm in the physical flow domain. The velocity vector fields were calculated using commercial PIV software (LaVision Davis 8.2.2).

Optical distortions, which were introduced by the curved walls of the test chamber, were corrected during post-processing. A calibration target, represented by a square grid with a node size of 1 mm and distance between nodes equal to 2 mm was placed in the plane of interest of the test chamber. The calibration images were processed in order to obtain the centroid locations of the grid points. A polynomial interpolation was subsequently used to warp the distorted PIV image of the undistorted one.

For each valve, the PIV data was collected in three data acquisition planes, located perpendicular to each of the commissure posts, as defined in Fig. 1c. The particle displacement data were used to calculate distributions of the velocity vectors $\underline{V}$, the out-of-plane vorticity ω_z , and the viscous shear stress (VSS), which is defined as follows:

$$\vec{\tau}_{xy} = \mu \left[\frac{\partial u}{\partial y} + \frac{\partial v}{\partial x} \right], \quad (2)$$

where u and v are the x- and y-components of the velocity vector $\underline{V}$ and μ is the kinematic viscosity of the fluid. At each data acquisition plane, five randomly selected statistically independent data sets, each containing 72 periods of a typical cardiac cycle, were acquired. The sampling rate at which the data was collected produced 172 phases over the typical period of the cardiac cycle. After the data from five independent data sets were combined together, each of the 172 phases of the cardiac cycle contained 360 instantaneous velocity fields, which were ensemble-averaged to provide a phase-averaged representation of the flow field.

3 Results

3.1 Hydrodynamic characteristics of the valves

The evolution of the EOA, transaortic pressure Δp , and RF as a function of number of cycles is shown in Fig. 2. The initial EOA for all three valves was equal to $1.93 \text{ cm}^2 \pm 0.05$. It can be seen that the EOA for valves A, B and C was very similar up to 20 million cardiac cycles. After 20 million cycles, the EOA for valves A and B started to decrease until it reached the value of approximately $1.54 \text{ cm}^2 \pm 0.05$ at 30 million cycles, and eventually decreased to the value of $1.17 \text{ cm}^2 \pm 0.05$ at 40 million cycles, which corresponds to a fail threshold defined in ISO 5840–2:2015 [25] for the tissue valves of an effective diameter of 23 mm. No variation in the value of EOA was observed between 40 and 50 million cycles. At the same time, the EOA of the control valve C decreased to $1.71 \text{ cm}^2 \pm 0.05$ at 50 million cycles mark and successfully passed the accelerated wear testing. The decrease in the EOA for valves A, B was followed by corresponding increase in the transaortic/mean pressure difference as well as regurgitant fraction, as shown in Fig. 2. The maximum value of RF did not exceed 10% and thus was in the “mild severity” bracket of aortic regurgitation [36]. At the same time, the increase in RF, in particular indicated the possible connection to the accelerated mineralization of the valve tissue and decrease of its performance.

3.2 Calcification of the valves: Location and amount of calcium deposits

Scanned samples of valves A and B provided good imaging of the calcium deposits, shown as bright-colored regions in the 3D reconstruction of the valves in Fig. 3, and revealed that the

Fig. 2 Effective orifice area (a), transaortic pressure (b), and regurgitation fraction (c) as functions of the number of cardiac cycles

absolute value of calcium volume in the specimens at 50 million cycles mark was equal to 2.7 mm³ and 2.2 mm³ in valve A and B, respectively. The presence of calcification deposits in case of the valve C, which was not subjected to accelerated calcification solution, was not detected.

3.3 Effect of calcification on the flow structure downstream of the valve

Quantitative flow visualization using the PIV technique was conducted at every 10 million cardiac cycles for the three valves: the valves A and B were subjected to accelerated, dynamic calcification protocol described above, while the control valve C was subjected to the identical hydrodynamic conditions and the number of cardiac cycles in a blood analog fluid without the calcifying solution.

The flow structure in the vicinity of the heart valve was analyzed in terms of the 2D patterns of the velocity and out-of-plane vorticity, which were measured in three data-acquisition

Fig. 3 3D reconstruction of the calcified heart valves. The calcium deposits are shown as colored regions

planes, corresponding to the three commissure posts of the valve leaflets defined in Fig. 1b. Moreover, distributions of the streamwise velocity component and the peak VSS along the cross sections of each data acquisition plane located at $Y = 20$ mm downstream from the valves provided insight into the deterioration of the hydrodynamic performance of the valves during the testing period. The evolution of the flow parameters was analyzed over three phases of interest in the cardiac cycle, which corresponded to the maximum ejection, the reduced ejection, and the closing phases of the systole, respectively. These phases are indicated as R_1 ($t/T = [0.02, 0.12]$), R_2 ($t/T = [0.12, 0.29]$) and R_3 ($t/T = [0.29, 0.34]$), respectively, in Fig. 4, which shows the variation of the flow rate Q , the aortic pressure (PAO) and the ventricular pressure (PVE) during a typical cardiac cycle.

The plots of Fig. 5 show distributions of the phase-averaged flow velocity and the contours of the out-of-plane vorticity ω_z at peak systole ($t/T = 0.12$) in the data acquisition plane corresponding to the commissure post CP_1 for the three valves under consideration after 50 million cardiac cycles. The three valves exhibited qualitatively similar flow structure characterized by a central jet issuing from the orifice of the valve along the centerline of the aorta. The maximum absolute values of the out-of-plane vorticity were observed in the shear layers that formed on the periphery of the jet, immediately downstream of the valve. The jet flow was accompanied by formation of a vortex ring, which corresponds to the counter-rotating vortices labeled in Fig. 5 as R and R'. Although the trileaflet valves under consideration had a symmetric geometry when fully open, geometric variations between the individual leaflets and their commissure points resulted in an asymmetric flow structure during systole, characterized by the pronounced deflection of the central orifice jet towards the left wall of the test section in the field of view illustrated in Fig. 5. As it can be seen in Fig. 5, the orifice is bounded by a rigid commissure post on the left side, while on the right side it is bounded by a flexible leaflet, whose deformation over the systole phase encouraged the deflection from the vertical axis of the central jet.

As mentioned earlier, the reduction in EOA was particularly pronounced in the case of the valves A and B, which were exposed to the calcification solution, as illustrated in Fig. 2. The plots of Fig. 6 show that this geometric degradation resulted in the increased streamwise velocity component of the central orifice jet during systole. Moreover, reduction of the EOA

Fig. 4 Typical variation of the flow rate Q , aortic pressure P_{AO} , and ventricular pressure P_{VE} as functions of time during a cardiac cycle

Fig. 5 Phase-averaged velocity vector fields and contours of the out-of-plane vorticity at the data acquisition plane corresponding to CP1 after 50 million cycles at peak systole ($t/T = 0.12$): **a** valve A, **b** valve B, **c** valve C

resulted in narrower jets produced by the valves A and B, compared to the case of the control valve C. Specifically, after 50 million cycles, the width of the central orifice jets, measured at $Y = 20$ mm in the data acquisition plane corresponding to CP₁, was equal to 20.4, 20.1, and 23.8 mm for the valves A, B and C, respectively. The corresponding peak streamwise velocity $u_{\max}$ was equal to 2.8, 2.8, and 2.2 m/s. The peak streamwise velocity values for the control valve C did not change significantly during the span of the test, with an increase in $u_{\max}$ of 10% observed between the initial condition and after 50 million cycles. In contrast, the valves exposed to the calcification solution exhibited a rapid increase in $u_{\max}$ during the initial part of the study (18% and 16% increase between 0 million and 30 million cycles for the valves A and B, respectively). Subsequently, the rate of change of the flow structure decreased, resulting in a minimal change in $u_{\max}$ between 30 million and 50 million cycles (13% and 15% increase for the valves A and B, respectively, in the data acquisition plane corresponding to CP₁.)

3.4 Effect of calcification on the VSS downstream of the valve at peak systole

During the evaluation of the streamwise evolution of the peak values of the VSS and the streamwise velocity component, it was observed that their respective maxima were attained at approximately $Y = 20$ mm downstream of the valve. This location was used to investigate the temporal evolution of these parameters.

3.4.1 Temporal evolution of the peak VSS during systole

In Fig. 7, the temporal evolution of the maximum absolute values of the VSS at $Y = 20$ mm is presented for the test valves A, B, and the control valve C, at the commissure posts CP₁, CP₂, and CP₃. The red dashed lines outline the temporal regions of interest R_1 , R_2 , R_3 , which were defined in Fig. 4. In the case of the control valve C, the variation of the peak VSS values over 50 million cycles at each commissure post was small compared to that of the valves A and B,

Fig. 6 Profiles of the streamwise velocity component corresponding to valves A, B and C, at $Y = 20$ mm during peak systole ($t/T = 0.12$) at **a** CP1, **b** CP2, and **c** CP3

which were exposed to the calcification solution. Initially (at 0 million cycles), the peak magnitude of the VSS of approximately 14 dyne/cm^2 was observed at each of the three commissure posts. A similar value of the VSS was observed in the cases of the valves A and B. For all three valves, a rapid increase in the magnitude of the peak VSS values was observed at $t/T = 0.07$, while a gradual decline in the magnitude of the peak VSS values was at $t/T = 0.12$ in the region R_2 , which corresponded to the deceleration of the flow.

In the case of the control valve C, the magnitude of the peak VSS over the period of 50 million cycles increased by 22%, compared to that at 0 million cycles, at CP_1 and CP_2 , and by 31% at CP_3 . A moderate increase in the magnitude of the peak VSS values between 20 and 50 million cycles was observed in the region R_1 at both CP_2 and CP_3 , while a significantly larger deviation from the initial peak VSS values was observed in the temporal region R_2 at CP_1 . This difference in the evolution of the VSS between the data acquisition planes corresponding to different commissure posts after 20 million cycles indicates a non-uniform deterioration of the leaflets of the control valve during this period.

Both valves that were exposed to the calcification solution (valves A and B) exhibited higher absolute maximum values of the VSS during systole, compared to the control valve C, at each considered data acquisition plane (CP_1 , CP_2 , and CP_3), as illustrated in the plots of Fig. 7. However, at CP_1 , shown in Fig. 7a, the absolute maximum VSS value corresponding to the valve A (22 dyne/cm^2) was higher than that corresponding to the valve B (19 dyne/cm^2), while at CP_2 , shown in Fig. 7b, the valve B produced the highest VSS value of 23 dyne/cm^2 , compared to the peak VSS value of 20 dyne/cm^2 in the case of the valve A.

Moreover, the rates of increase of the peak VSS values as functions of the number of cardiac cycles corresponding to the valves A and B had different trends at CP_1 and CP_2 . In particular, at CP_1 , the peak VSS values corresponding to the valve A increased by 30% between 10 and 30 million cardiac cycles and by 22% between 30 and 50 million cycles. The peak VSS values corresponding to valve B increased by 28% between 10 and 30 million cardiac cycles and by 16% between 30 and 50 million cycles.

When the same parameters were measured at CP_2 , shown in Fig. 7b, the peak VSS values corresponding to the valve A increased by 30% between 10 and 30 million cardiac cycles and by 10% between 30 and 50 million cycles. The peak VSS values corresponding to valve B increased by 40% between 10 and 30 million cardiac cycles and by 16% between 30 and 50 million cycles.

At the data-acquisition plane corresponding to CP_3 , shown in Fig. 7c, although the absolute maximum VSS values were similar to both valves A and B (22 dyne/cm^2 , corresponding to 50 million cardiac cycles,) the rates of increase of the peak VSS as the function of the number of cardiac cycles was different. The peak VSS values corresponding to the valve A increased by 21% between 10 and 30 million cardiac cycles and by 23% between 30 and 50 million cycles. The peak VSS values corresponding to valve B increased by 36% between 10 and 30 million cardiac cycles and by 23% between 30 and 50 million cycles.

4 Discussion

The present study characterized the process of in vitro, dynamic calcification of the porcine aortic heart valves, measured the progressive decrease of the EOA and subsequent increase of the mean transaortic pressure Δp , and documented the connection of the in vitro, dynamic calcification process with subsequent changes in the flow field. This study complements and

Fig. 7 Temporal evolution of maximum VSS for valves A, B, C at $Y = 20$ mm during the systole phase at **a** CP_1 , **b** CP_2 , and **c** CP_3

extends the previous studies [1, 5, 9, 10, 14, 17, 26, 37–39], which have demonstrated the importance of modeling the *in vitro* calcification process as a viable tool in assessing the failure of clinical bioprosthetic heart valves due to premature calcification.

It was not clear what mechanism was responsible for the minor decrease of the EOA for the control valve C. It was assumed that the exposure of the control valve to the glycerin/saline blood analogy solution during PIV data acquisition might be partially responsible for that phenomenon. There are several reports, such as [40–42], in which it was speculated that glycerin may potentially be absorbed into the tissue leaflets, increasing their stiffness. A small uni-axial or bi-axial type testing of the leaflets to characterize the changes in stiffness and flexibility, which is beyond the scope of the current study, would be very helpful in quantification of these effects.

The volume of the calcium deposits was in the lower part of the range reported in earlier studies [1, 4, 37]. However, the location of the calcium deposits, which was observed predominately in the outflow regions and near the commissure posts, was in line with those reported in the abovementioned studies. Based on the hydrodynamic assessment of the valves as well as on the location and volume of calcium deposits, it can be inferred that the decrease of the EOA was predominately due to the process of in-tissue calcification [6, 9, 19] that led to the overall tissue stiffening rather than due to flow obstruction caused by calcium deposits, which was indirectly demonstrated through hydrodynamic testing and quantitative flow visualization. This observation is confirmed by another study, conducted on a transcatheter heart valves subjected to a controlled transcatheter crimping, following the identical accelerated calcification protocol [9].

In this study, two test valves A and B subjected to calcification solution were tested alongside one control valve C. All three valves were of identical type and size. Although larger statistical samples were not feasible for this time-consuming study, the following observations of the variation observed between the two treated valves indicate that the hydrodynamic characteristics of both valves (A and B) that were exposed to the calcification solution deteriorated to a similar state at 50 million cycles, but there were differences in the rates of deterioration of the hydrodynamic performance between the valves A and B. Specifically, the measured peak VSS values corresponding to the valve B increased more rapidly, particularly at the beginning of the testing period. There is also a possible impact of valve-to-valve variability on the results, which was manifested through a non-uniform deterioration of the leaflets during 50 million cycles period.

4.1 Limitations of the study

In this study, we considered the correlation between long-term *in vitro* dynamic calcification and abnormal flow patterns associated with three porcine bioprosthetic heart valves. The effort was made to replicate, as closely as possible, the calcification process, while still maintaining the feasibility of the method for the *in vitro* testing. The developed test protocol closely followed the standard durability test method and the hydrodynamic assessment of the market-approved heart valves ISO 5840–2:2015 [25].

There are several limitations to this study. Due to limited availability, only three test samples were used, with one valve being used as a control device. Thus, the results are limited to the particular type of test valves and their physical dimensions. Small number of test samples inherently indicates the lower statistical significance of the results presented in the current study. However, the results presented herein correlated well with previously published studies [1, 11, 18, 26]. In addition to this, an effort was made to make sure that the main quantities reported in the study are statistically accurate and were derived from an appropriate

number of samples. The increase of the number of samples to at least five would allow predicting the premature calcification behavior of the family of porcine surgical heart valves more confidently.

With respect to the tissue type used in the current study, the majority of TAVI valves are made of bovine pericardial while the current study was aimed at the porcine aortic valves, as such the applicability of the results might be limited to the devices such as Symetis Acurate TA™ and JenaValve™. While there are some underlying differences in the calcification processes between porcine aortic leaflet and pericardial leaflets, in the early works by Schoen [6, 43], it was demonstrated that there is a strong similarity in morphological progression and kinetics of calcification between bovine pericardium and porcine aortic valves.

In addition, the test section of flow loop was simplified, and the aortic root geometry was not replicated. Several studies indicate that the interaction of the vortices that form in the aortic sinuses with the flow are essential for the calcification process. The lack of sinuses did indeed influence the fluid dynamics both near and downstream of the valve. There are two main reasons, however, that ultimately lead to the decision to avoid using sinus geometries in the current study: (1) All of the commercial heart valve durability testers, such as Bose ElectroForce, BDC VDT system, or ViVtro HiCycle do not have root sinuses in their standard configuration. Moreover, ISO 5840–2:2015 [25] does not require the presence of such chamber modifications for proper durability testing. (2) For the quantitative flow visualization in the chamber with sinuses, one would need to match index of refraction of the fluid with that of the chamber material in order to negate spherical and cylindrical optical distortions. Typically, a mixture of NaI, Glycerin and H₂O is used, which is not compatible with tissue in a bioprosthetic valves. (3) As this study represents an attempt to model a dynamic calcification process, some simplifications are necessary to ensure repeatability of the results. However, along with limitations, the simplified geometry of the experimental setup had significant advantages. In particular, the simplified setup allowed systematic examination of the flow features inherent to the heart valve, without the complex interactions with the aortic root.

Moreover, the test section was made of a rigid material. Although compliance of the aorta was modeled experimentally by including a confined volume of air in the flow loop, the setup did not allow investigation of the effect of compliance of the aortic root on the magnitude and evolution of the flow patterns.

During the accelerated calcification process, the samples were not continuously submerged into calcification solution over the entire duration of the test. After every 10 million cycles, the valves were temporarily removed from the solution in order to perform hydrodynamic assessment and quantitative flow visualization. At that time, the valves were subjected to the glycerin/saline blood analog fluid, which density, viscosity, and pH is different from that of calcification solution. The low volume of calcium deposits compared to that in the previous studies [1, 37] and longer times to achieve the equivalent amounts of calcification might be attributed to these interruptions of the calcification process, as well as due to the anticalcification treatment of the test valves by an agent containing ethanol and Tween-80, details of which were not disclosed by the manufacturer.

5 Conclusions

In this study, two aortic heart valve prostheses were subjected to the accelerated wear testing over 50 million cycles in the artificial calcification solution with the main goal to reveal a

possible correlation between calcification of the leaflets and development of abnormal flow features downstream of the valves, such as elevated values of the viscous shear stress (VSS) and the streamwise velocity. The presence of calcium deposits was confirmed through the micro-computed tomography imaging, which revealed the absolute value of calcium volume in the two tested specimens of 2.7 mm^3 and 2.2 mm^3 .

The calcified valves failed the ISO 5840–2:2015 [25] criterion for hydrodynamic performance after 40 million cardiac cycles. The valves exhibited a rapid decrease of the effective orifice area EOA as well as a rapid increase in the peak values of VSS and the average streamwise velocity after 20 million cardiac cycles. Specifically, the overall decrease in the EOA from 1.9 cm^2 to 1.2 cm^2 was observed, which was subsequently followed by an increase in the transaortic pressure Δp from 12.1 to 22.7 mmHg. The decrease in the EOA was also associated with the increase of RF from about 1 to about 10%. While there are still not enough clinical data to indicate the alarming frequency of combined presence of stenosis and regurgitation, particularly in the older patients, as the number of older patients increases every year, the number of cases where stenosis and regurgitation present simultaneously might increase as well. In contrast, only a minor change in the EOA, Δp and RF was observed in the case of the control valve, which was not exposed to the calcifying solution during 50 million cardiac cycles.

The quantitative flow visualization revealed changes in the evolution of the peak VSS values as well as peak magnitude of the streamwise velocity as functions of the number of cardiac cycles. A little variability in the peak values of VSS and magnitude of the streamwise velocity was observed between three data acquisition planes, which suggested a lack of strong spatial preference in the orientation of the mineralization process in the current study. The decrease in EOA was subsequently followed by the rapid increase in the maximum streamwise velocity of the central orifice jet up to approximately 2.8 m/s and progressive narrowing of the velocity profile for valves that were exposed to the calcifying solution. The magnitude of the peak VSS values was observed just after peak acceleration phase of the cardiac cycle, at $t/T = 0.12$, which is associated with the maximum ejection of the fluid from the ventricle. In the case of the calcified test valves, an overall increase of the maximum VSS between 44% and 52% observed over the 50 million cardiac, depending on the data acquisition plane, in which the measurement was performed. The corresponding increase in the peak VSS values in the case of the control valve was between 22 and 30%.

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Compliance with ethical standards

Conflict of interest Oleksandr Barannyk has been a postdoctoral fellow at ViVtro Labs Inc. under MITACS Accelerate research grants. Robert Fraser is an employee of the ViVtro Labs Inc.

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