ORIGINAL ARTICLE

Developing the Tissue Engineered Heart Valve – a Descriptive Hemodynamic and Ultrasound *in Vitro* Characterization Study of Heart Valves in a Bioreactor

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ABSTRACT

The inherent limitations of current heart valve substitutes create the premise for the Tissue Engineered Heart Valve (TEHV), considered the perfect substitute. We aimed to compare *in vitro* hemodynamic performances of our TEHV, the conventional prosthetic valve and similar porcine valves, by ultrasonography and geometry resulting in six valve models analysis. In a bioreactor, pulmonary and aortic physiology were replicated thus hemodynamic characteristics were tested. Using ultrasound, transvalvular pressure gradients and flow were measured and used to calculate their valvular functional area (VFA) and using a high-speed camera, the geometric peak opening area (GOA) was assessed. The obtained results were normalized to the diameter of the biological prosthesis in order to increase the measurement's accuracy. The ultrasound revealed normal function of all valves and physiologic transvalvular pressure gradients. The TEHV scaffold revealed absence of laceration or dehiscence, and performances in accordance with the control prostheses. The GOA was facile to obtain and the normalized values proved to be greater than the calculated functional area in all analyzed cases and the peak opening areas resulted lesser for the aortic conditions for all six used valves prototypes. To our knowledge, this is the first study to use bioreactors, for *in vitro* evaluation of heart valves.

Keywords: tissue engineered heart valve, heart valve bioreactor, *in vitro* ultrasound, regenerative medicine, heart valve substitute.

REZUMAT

Limitările inerente ale actualilor substituenți valvulari creează premisele pentru valvele cardiace obținute prin bioinginerie, considerate substituenți ideali. Am dorit comparația performanțelor hemodinamice *in vitro* a valvei noastre, protezelor convenționale și valvelor similare proaspete porcine, prin ultrasunografie și geometrie, rezultând astfel analiza a șase modele valvulare.

Într-un bioreactor, condițiile pulmonare și aortice au fost reproduse, testând astfel caracteristicile hemodinamice. Ecografic, s-au măsurat gradienții transvalvulari și debitul fiind folosiți pentru calcularea ariei valvulare funcționale (AVF) a acestora iar folosind o cameră de mare viteză, a fost evaluată geometric aria de deschidere (GOA). Ecografia a relevat funcția normală a tuturor protezelor și gradienți transvalvulari fiziologici. Scaffold-ul obținut de noi a prezentat performanțe comparabile cu protezele utilizate ca și control fără a se evidenția semne de lacerații sau traumatisme. GOA a fost un parametru care a fost cu ușurință calculat, iar valoarea normalizată a sa s-a dovedit a avea valori mai mari decât aria funcțională calculată iar la comparația celor două regimuri hemodinamice, cea aortică a prezentat valori mai reduse în toate situațiile. Revizuirea literaturii ilustrează acest studiu ca primul care utilizează bioreactoare, pentru evaluarea *in vitro* a valvelor cardiace.

Cuvinte cheie: valve cardiace obținute prin bioinginerie, bioreactor de valve cardiace, ecografie *in vitro,* proteză valvulară.

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INTRODUCTION

Heart valves play a key role in cardiac function, blood circulation and hemodynamic. Through their opening and closing motion they establish the unidirectionality of blood flow between the chambers of the heart and the main vessels. Thus, when affected by disease, their malfunction leads to heart failure or even death. Current therapies for amending valve failure are based on surgical or interventional repair or replacement[1]. Although many improvements have been recently observed in these replacement options, in terms of the materials used and their proprieties[2-4], the limited durability of the biological valves and the inherent thrombogenicity requiring lifelong anticoagulation therapy of the mechanical valves make both these options imperfect and, in practice, at many times just temporary solutions[5]. In this regard, valvular replacement in pediatric patients represents an even more demanding challenge, frequently requiring consecutive surgical interventions in order to match the valvular prosthesis size to the constantly growing heart[6].

Bioengineering aims to obtain living tissues and organs *in vitro,* using tissue engineering and regenerative medicine[7-10]. The important function played by heart valves and the shortcomings of current available therapeutic replacements make them ideal candidates for tissue regeneration research. Thus, multiple research groups[11-14] have already investigated and published on different stages of the process of obtaining a Tissue Engineered Heart Valve (TEHV).

In order to produce a TEHV, autologous cells are seeded on an acellular scaffold based on extracellular matrix and this hybrid structure is subsequently tested, pre-exposed and pre-conditioned to prespecified *in vivo* conditions. These procedures are performed in bioreactors, which are purposely built laboratory systems used to recreate different physiologic conditions and test bioengineered tissues or organs. To date there is a large variety of these machines produced[15-17], some specifically designed to test and pre-condition a specific TEHV component[18,19], while others are built to test the entire valvular assembly[20,21]. Using bioreactors, TEHV are cyclically exposed to the pulsatile flow and the strain produced by each opening and closing cycle in a strictly controlled environment with preset pH, O_2 concentration, fluid viscosity and temperature. TEHV functional evaluation during exposure in the bioreactor is important, because it simulates *in vivo* conditions. While some parameters like opening frequency and transvalvular pressure or flow can be easily monitored, other important functional measurements are not readily available.

The aim of our study was to descriptively characterize the hemodynamic performance of different natural and manufactured heart valves in comparison to our TEHV scaffold, using ultrasound *in vitro,* in specific cardiac physiology conditions simulated in the laboratory using a heart valve bioreactor.

MATERIALS AND METHODS

This work is part of a research project that was approved by the local Ethics Committee. Using a Valve Bioreactor (Aptus Bioreactors, Clemson, South Carolina, USA – www.aptusbr.com), six types of heart valves were tested while under conditions replicating the aortic and pulmonary physiology. These were divided into three groups. The first group consisted of our TEHV scaffold, specifically aortic and pulmonary porcine valves processed in the laboratory through decellularization. The second group contained currently used replacement valves, specifically a three-dimensional printed version of standard mechanical bileaflet prosthesis and a standard bovine pericardium derived bioprosthesis. Finally, the third group consisted of freshly harvested porcine aortic and pulmonary heart valves to serve as a standard comparator.

All heart valves had their functional hemodynamic parameters assessed in a controlled setting in the laboratory, using ultrasound examination, while exposed to *in vivo* conditions specific for the right and left heart functioning regimens, which included fixed systolic and diastolic pressures, heart rate, stroke volume, and flow rates. Using a commercially available ultrasound system, multiple opening and closing cycles were recorded and a comprehensive analysis was performed on each model. The functional opening area of the valves was calculated using data derived from the ultrasound measurements and Gorlin's formula[22],

$$Valve\ Area\ (in\ cm^2) = \frac{Cardiac\ Output\ (ml/min)}{Heart\ rate\ \left(\frac{beats}{min}\right) x Systolic\ ejection\ period\ (s) x 44.3 x\ square\ root\ (mean\ Gradient - mmHg)}$$

as presented below. Also, using a high-speed camera (iDS Imaging, Obersulm, Germany), consecutive snapshots were taken during multiple opening cycles, in order to evaluate the valve's movement and anatomical opening area, which was calculated using ImageJ software (U. S. National Institutes of Health, Bethesda, Maryland, USA).

1. The heart valve prostheses models

Our TEHV scaffolds consist of decellularized porcine aortic or pulmonary valves, obtained by processing fresh porcine hearts from healthy animals. The organs are procured from a local slaughterhouse shortly after the animal's death; they are harvested using a technique that preserves both the aortic and the pulmonary trunks and afterwards are rapidly transported on ice to the laboratory. Then, they are meticulously inspected in order to exclude any anatomical or pathological abnormalities which, if found, lead to the discarding of the organ.

Through careful dissection, the aortic[23] and pulmonary valves are isolated and harvested. Afterwards, the valves undergo the process of perfusion decellularization in the PDCeller heart valve decellularization device (Aptus Bioreactors, Clemson, South Carolina, USA), which takes place over many days. The protocol consists of successive exposure of the valves to a mix of detergents, enzymes and alcohol for a duration of 26 days for the aortic valve[24], respectively 10 days for the pulmonary valve[25] with a final phase of chemical sterilization using peracetic acid. These treatments were applied to the fresh valves under a pressure gradient (the minimal value that produced expansion of the valves arterial walls – 20 mmHg for the pulmonary valves, 50 mmHg for the aortic valves a process performed in order to facilitate the diffusion and potentiate chemical activity The final success of the procedure is evaluated by histological sampling and DNA extraction. In order to prepare the TEHV scaffolds for bioreactor exposure, they are then fixed with a 3.0 polypropylene suture to the fixing system of the bioreactor (Figure 1).

For the valvular prostheses comparisons, which were part of the second group, we chose a commercially available aortic stented bioprosthesis, with a stent composed of a cobalt and chromium alloy and the leaflets composed of bovine pericardium covered with polyester cloth (Edwards Lifesciences, Carpentier – Edwards, Perimount Magna, size 19), which was fixed to the fitting ring of the bioreactor with a 3.0 polypropylene suture at commissural level. Due to the large variety of commercial valvular prosthesis which we could not cover, we opted to create a 3D printed version of a standard mechanical bileaflet prosthesis (St Jude Medical Heart Valve), with the size chosen in order to best match the fitting ring assembly of the bioreactor (Figure 1).

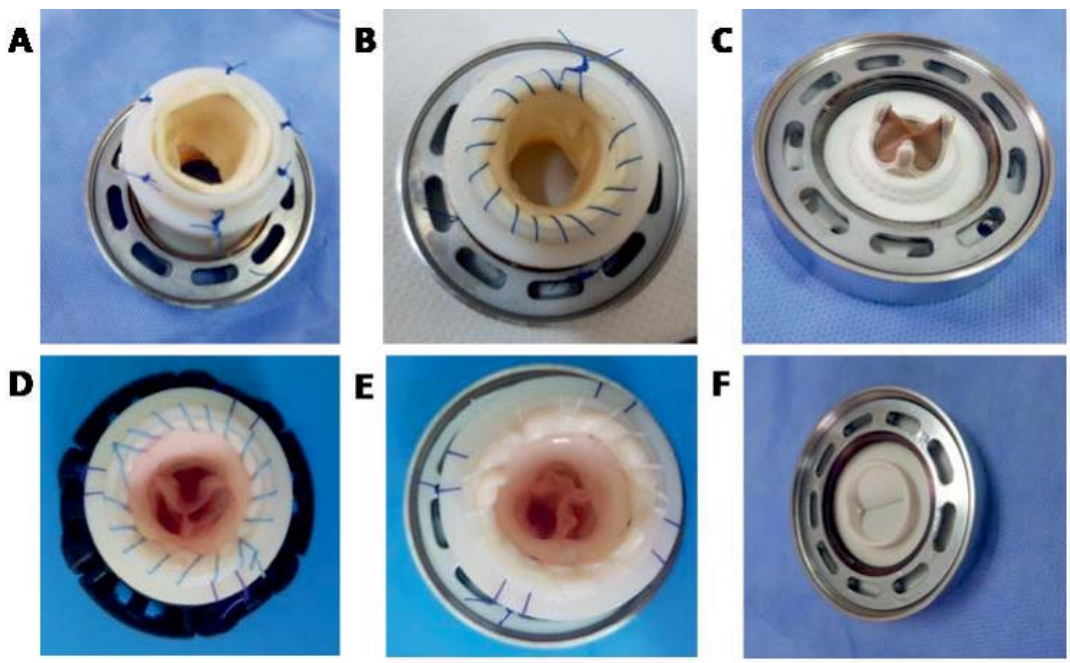

Figure 1. Tested valvular models – A – Decellularized porcine aortic valve; B – Decellularized porcine pulmonary valve; C- Biological prosthesis; D – Fresh porcine aortic valve; E – Fresh porcine pulmonary valve; F – mechanical valve.

The freshly harvested aortic and pulmonary porcine valves, used as controls in the third group, were first isolated, then their coronary arteries were ligated with a 2.0 coated braided polyester suture and afterwards they were sutured to the fixing system of the bioreactor. In order to preserve the characteristics of freshly harvested tissues, all procedures and examinations for these valves were performed in the first four hours after harvesting (Figure 1).

The prostheses sizes were geometrically evaluated by their diameter. The biological prosthesis measured 1.9 cm, 2.0 cm the fresh and the decellularized valves, respectively 2.1 cm the mechanical valve. In order to increase the comparison accuracy, the results were normalized to the diameter of the biological prosthesis.

2. The bioreactor

Using a dedicated heart valve bioreactor (Figure 2A), aortic and pulmonary flow regimens were reproduced successively by adjusting the bioreactor's Aptus PhysioTM software different settings and physical components. The commercially-available bioreactor is an upgraded version of one previously published (26). The pulmonary regimen consisted of a systolic/diastolic pressure range between 15/6 and 25/5 mmHg, with a mean of 20/5 mmHg, an average stroke volume of 60 mL and a work rate of 70 cycles/min.

For the aortic condition, the pulmonic compliance chamber components (tall glass tube) were exchanged for the aortic conditions chamber components (not pictured) and a clamp was added between the compliance chamber and the reservoir, having the role to produce a resistance to the flow. Above 100 mmHg pressures were generated through increasing the peripheral resistance. Aortic conditions were simulated with a systolic/diastolic pressure range between 120/77 mmHg and 123/100 mmHg, with a mean of 121/93 mmHg, a work rate of 70 cycles/min and an average stroke volume of 79 mL and an opening period of 300ms (Figure 2).

3. Ultrasound examinations

Ultrasound examinations were performed in the laboratory using a commercially available ultrasound system (Logiq E, GE, Boston, MA, USA) and a 4.0 MHz phased array transducer. Using conventional two-dimensional 2D imaging we evaluated the anatomy, integrity and movement of the prostheses' components, including the annulus, sinuses, leaflets and walls. Also, the prostheses' opening and closing mechanisms were examined, in both the longitudinal and transversal planes. Using Color Doppler imaging, the functionality of the prostheses was analyzed by evaluating signs of valvular stenosis, through the presence or absence of laminar flow, or of valvular regurgitation, manifested by the appearance of a regurgitant flow. Lastly, with the help of Pulsed-Wave Doppler imaging, transvalvular velocities and pressure gradients were measured. These parameters were used to calculate functional area using Gorlin's formula.

The ultrasound measures performed by the same operator, a cardiologist specialized in transthoracic and transesophageal echocardiography.

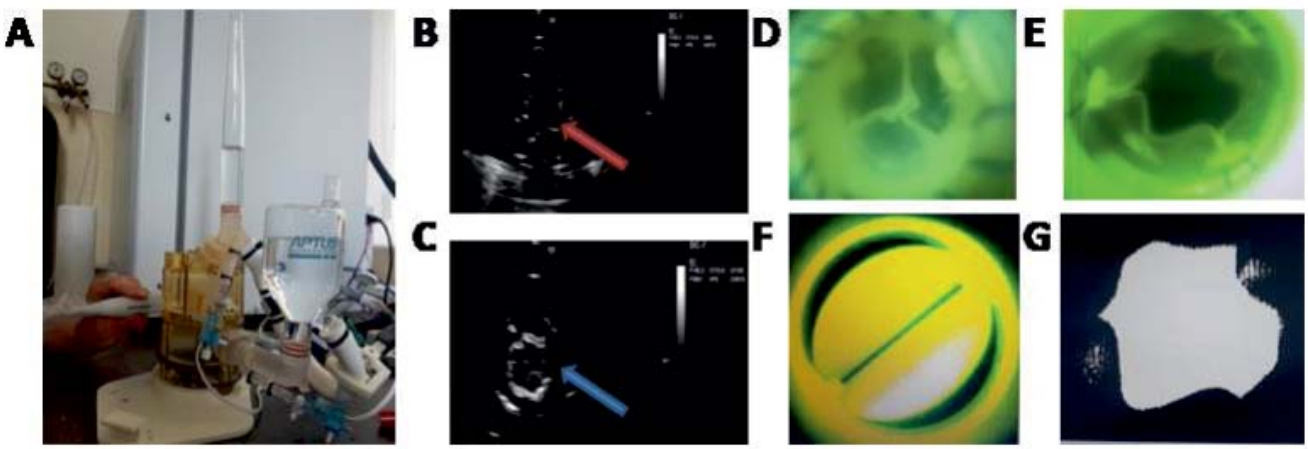

Figure 2. Ultrasound and photographic examination – A – general aspects of the heart valve bioreactor for pulmonary conditions and transversal plane technique, the ultrasound probe is marked in green; B – ultrasound aspect of longitudinal plane examination – the leaflets are marked with the red arrow; C- ultrasound view of transversal plane examination – the central coaptation of cusps is marked with the blue arrow. D – Snapshot of a decellularized porcine aortic valve; E – Snapshot of a biological prosthesis; F – Picture editing of the mechanical prosthesis used to calculate the opening area; G – Software evaluation of the opening are of a biological prosthesis.

4. Image capture and processing

Simultaneously with the ultrasound examinations, the prostheses' movements were recorded with a high-speed camera (Figure 2 – D;E). Through a dedicated video-photography processing software (DVDVideo-Soft - Free Video to JPG Converter), successive frames were extracted. In ImageJ software, the frames were transformed in black and white binary images in order to outline the edges of the opening area. Subsequently, the ImageJ software was used to approximate the geometric opening area by comparing the measurements to an inputted pre-measured value (Figure 2 – F; G). In order to increase the precision, normalization of valvular areas to the standard biological prosthesis was performed. To meet this purpose, the calculated opening area was divided by the measured valve diameter (0.19 cm for the biological valve, 0.2 cm for the fresh and the decellularized valves respectively 0.21 cm for the mechanical valve) and multiplied by the standard stipulated diameter of the biological prosthesis (0.19 cm).

RESULTS

1. Decellularization evaluation

Histology and DNA extraction revealed cells absence and integrity of the extracellular matrix without migration lines in agarose electrophoresis[24,25].

2. Morphological assessment

Standard biplane ultrasound examination revealed the integrity of all the TEHV components, with no signs of lacerations or valvular rupture. The annulus presented a homogenous look, with normal echogenicity and no nuclei suggestive of calcification present. The arterial wall appeared smooth, with no intimal thickening and no signs of atheroma and the semilunar sinuses presented normal features with no signs of dilatation. Regarding the aortic decellularized roots, the coronary ostia appeared physiological without any signs of dissection and the valves themselves were thin, mobile and presented a fluid opening and closing movement. In all, no signs of abnormal morphology or function were determined. The conventional valvular prostheses group revealed adequate mobility and opening of the leaflets for the mechanical valve and a perfect alignment of the closed discs, however it must be noted that the ultrasound images contained multiple artifacts due to the metallic structure of the fitting system of the valve. Regarding the biological valve, the evaluation revealed a good opening of the valve and complete and efficient closure. The pericardial leaflets appeared mobile and thin and, as was the case for the mechanical valve, numerous imaging artifacts were recorded due to its metallic component. The freshly harvested heart valves presented physiological behavior, normal echogenicity of all components and no signs of calcifications or atheroma.

Table 1. Ultrasound examination results in pulmonary and aortic conditions

Pulmonary conditions				
	TEHV Scaffold	**Fresh Pulmonary valve**	**Biological valve prosthesis**	**Mechanical valve prosthesis**
V max (cm/sec)	328 ± 85	355 ± 25	260 ± 41	181 ± 39
V mean (cm/sec)	127 ± 28	134 ± 10	124 ± 19	111 ± 7
P max (mmHg)	55.12 (19.00-60.78)	50.86 (45.98-57.63)	26.89 (18.09-38.25)	13.60 (7.36-20.40)
P mean (mmHg)	11.82 ± 5.42	12.26 ± 1.58	9.04 ± 2.47	4.42 ± 2.10
VTI (cm)	45.54 ± 9.83	47.46 ± 2.09	42.23 ± 7.23	30.71 ± 7.54
Functional area (cm^2)	1.14 (1.07-1.92)	1.24 (1.17-1.29)	1.43 (1.24-1.69)	2.09 (1.68-3.03)
Aortic conditions				
	TEHV Scaffold	**Fresh Aortic valve**	**Biological valve prosthesis**	**Mechanical valve prosthesis**
V max (cm/sec)	431 ± 64	469 ±143	368 ± 60	399 ±121
V mean (cm/sec)	130 ± 33	163 ± 43	132 ± 21	161 ± 8
P max (mmHg)	68.27 (60.28-100.75)	75.37 (46.78-158.50)	64.71 (33.00-68.08)	85.60 (23.83-101.50)
P mean (mmHg)	9.42 (8.08-22.00)	14.69 (9.60-30.67)	13.83 (7.00-14.51)	17.15 (7.10-18.00)
VTI (cm)	45.99 ± 11.32	51.13 ± 15.84	46.25 ± 7.64	48.08 ± 12.94
Functional area (cm^2)	1.28 ± 0.27	1.14 ± 0.34	1.32 ± 0.26	1.26 ± 0.34

Values are expressed as mean ± standard deviation for normal distribution and median (25^{th}-75^{th} percentile) for non-normal distribution.

3. Functional evaluation

Examination with the color Doppler mode revealed laminar flow through the TEHV scaffold, with no signs of reverse flow. The mechanical prosthesis presented two minimal eccentric regurgitant jets. The biological prosthesis presented turbulent flow and no signs of valvular regurgitation. The fresh aortic and pulmonary valve revealed laminar flow and no regurgitation.

Forty-eight valve opening and closing sequences were examined using Pulsed-wave Doppler analysis. The flow tracings were manually delineated and for each type of valve, five parameters were recorded and measured: transvalvular peak velocity (V_{max}), transvalvular mean velocity (V_{mean}), transvalvular peak pressure gradient (P_{max}), transvalvular mean pressure gradient (P_{mean}) and velocity-time integral (VTI). For each valve, five consecutive opening cycles were recorded, and all parameters were measured for every cycle. These values were then used to calculate a mean for every parameter. All these measurements were performed first with the bioreactor set for pulmonary conditions, then with the settings changed for aortic conditions, thus in all a total of 96 opening cycles were analyzed (Table 1).

3. Video and image measurements

For each valve six opening and closing cycles were recorded. Motion clips were analyzed by extracting from each cycle at least 16 different still images, in order to evaluate the opening and closing motion. From these, on average, thirteen consecutive frames at one ms time difference were extracted, with an end total of 1248 images analyzed, and for each the opening area was calculated using the specific software noted above. From these measurements, the maximum opening area value was chosen for each cycle and a mean value was calculated for each valve by averaging the six cycles. Dynamic evaluation of the valves revealed a smooth and full opening of the leaflets and a subsequent complete closure for all valves (Table 2).

DISCUSSIONS

The aim of our study was to evaluate the function of our TEHV scaffold in comparison to other valves *in vitro,* using a bioreactor to simulate cardiac physiology, specifically the aortic and pulmonary physical conditions. We focused our study on the behavior of the semilunar valves scaffolds due to their less complex anatomical and morphological organization and secondly due to the fact that our field of expertise is focused towards this domain. Ultrasound imaging can be used to calculate the anatomical opening area of a valve by planimetry, a standard method for measuring valvular area used for *in vivo* examination[27]. However, this was not considered a feasible option in our case because of the presence of the fitting structures of the bioreactor, which are metallic and create acoustic shadows that do not allow for a precise measurement of the opening area[28]. For this purpose, considering our *in vitro* examination conditions, we opted for a visual assessment of the anatomic area using photography and a dedicated software to select the peak opening area of each valve and prosthesis.

In order to perform the functional assessment, we used the ultrasound examination to calculate transvalvular velocities and pressure gradients, which were later used to calculate functional valvular area. The most common *in vivo* echocardiography method used for evaluating functional valve area is the continuity equation[29], which is a based on the principles of fluid dynamics and uses as variables the measured blood flow in the adjacent ventricular outflow tract and the peak transvalvular velocity. In our case we could not use this equation, because of the design of the bioreactor, so we opted to apply the Gorlin formula, of which requires direct measurements of transvalvular flow, because all of the variables used in the formula could be programmed using the bioreactor software. While this formula is frequently used in current practice when doing invasive pressure measurements, it does present some shortcomings. A published *in vivo*

Table 2. Video analysis in aortic and pulmonary conditions

	TEHV scaffold	Fresh valve	Biological valve prosthesis	Mechanical valve prosthesis
Normalized pulmonary conditions peak opening area (cm^2)	2.916±0.102	3.459±0.099	2.018±0.013	2.920±0.106
Normalized aortic conditions peak opening area (cm^2)	1.614±0.046	3.330±0.144	1.818±0.087	2.756±0.015

Values are expressed as mean ± standard deviation for normal distribution and median (25^{th}-75^{th} percentile) for non-normal distribution.

echocardiography study compared it to planimetry measurements and found variations in the calculated values when used in situations with acute changes of transvalvular volume, due to its dependence on blood flow[30]. Another small study revealed that when used for prosthetic heart valves (both biological and mechanical), applying the Gorlin formula resulted in biased over- or underestimated values[31] in comparison to hemodynamic and planimetric measurements. However, because we studied normally functioning valves and prosthesis, confirmed by the color Doppler examination, our goal was not to precisely determine the functional area, but to characterize our TEHV scaffold and other valves in standardized conditions in the bioreactor. Thus, the parameters used in Gorlin's formula were identical for every valve, which eliminated any result variability that can be caused by the equation itself.

In our study, with the bioreactor set to the aortic settings, the hemodynamic performance of our TEHV scaffold presented physiologically with normal morphology and function along with the other investigated valves. This shows that the preparation process of our TEHV scaffold does not affect the tissue properties of the valve and results in a structure with the same hemodynamic profile to a normal valve or bioprosthesis or mechanical prosthesis, in the high-pressure conditions of the aortic circulation.

Through its design, the bileaflet mechanical prostheses exhibits one central opening and two other smaller side openings[32], which produce a greater resistance to flow than normal semilunar valves, especially when working in a low-pressure system. Thus, they have an inherently different hemodynamic performance than any other native valve or bioprosthesis, in the low-pressure conditions exhibited in the pulmonary circulation, with reduced transvalvular velocities and pressure gradients due to the reduced mechanical resistance. Also, the Gorlin equation uses the mean transvalvular pressure gradient to calculate functional area, which explains the differences related to this parameter when comparing the mechanical prosthesis to the other types of valves.

The video analysis resulted in different peak opening area of the TEHV scaffold and all the other valves in the aortic conditions and pulmonary conditions. However, studies have shown that the geometric opening area of a valve is different than the functional opening area, mostly depending on the shape of the valve inflow area, and that the later is the more important measure of valve function[33,34]. Moreover, the transvalvular pressure gradient is not constant throughout the opening cycle which makes the functional valve area a more accurate measure of the mechanical work performed in order to create flow through the valve, than the geometric measurement of the peak opening area[35].The difference between functional and geometric valve area is further influenced in mechanical prostheses by the loading conditions, as observed in our study, where the peak opening area for this type of valve was similar in both the aortic and pulmonary settings (2.8 vs. 2.9 cm^2), whereas the functional area differed (1.26 vs. 2.09 cm^2) between the two regimens. Nevertheless, geometric orifice area data is valuable to obtain additional direct comparisons of the maximum opening area between multiple valves or valve types.

STUDY LIMITATIONS

The main limitation for our study was the limited numbers of valvular prostheses examined. Due to the large variety of these in existence, a full coverage could not be provided, so one representative biological and mechanical valve of similar size to our TEHV scaffold was chosen for each category. Also, the aim of the study was to compare the functional behavior of these valves *in vitro*, not to examine every possible prosthesis. Therefore, a single valve got us a sample of size over six valves. Further research on this topic should be performed in order to comprehensively evaluate the function of different sized valves of different type in the bioreactor. Even though the different types of valves were matched in size, slight differences could still have existed, which could have interfered with the study results. Moreover, a further limitation of the study could be represented by the usage of the 3D printed version of the bileaflet mechanical valve, that could present slightly different hemodynamic to an actual mechanical heart valve prosthesis because of the different fabrication materials. We choose this option in order to have a valve with a tight connection to the metal fixing ring of the bioreactor, necessary for our experiment.

Another limitation regarding functional assessment was the manual measurement of the ultrasound parameter by the operator, which could produce variations in Doppler velocities. In this regard a standard examination protocol was implemented for all the valves and serial averaged repeated measurements were performed in order to reduce possible measuring errors. Regarding the geometric measurements, by normalizing the area, this limitation was eliminated.

This paper presents only a restrictive descriptive analysis of the TEHV scaffold hemodynamic performances. In order evaluate and to compare its behavior to the present valvular substitutes further studies with an extensive number of scaffolds being investigated are required.

CONCLUSIONS

In this *in vitro* ultrasound study in the bioreactor, our TEHV scaffold performed physiologically with no evidence of regurgitation or stenosis. Morphologically, all valvular anatomical components presented normal movement and function without signs of laceration or dehiscence, in accordance with the control prostheses, freshly harvested valves, the bioprosthesis and the mechanical prosthesis in the aortic regimen.

Normalized values of the peak opening areas resulted lesser for the aortic conditions for all six used valves prototypes. The peak opening areas proved to be grater than the calculated functional area in all analyzed cases.

This study bioreactors, which recreates *in vivo* conditions, for *in vitro* comparison of heart valve types, highlights its valuable applicability for a better quantification of valvular function prior to implantation. Also, this work certifies the utility of the bioreactor in regenerative medicine heart valve research.

Compliance with ethics requirements:

The authors declare that all the procedures and experiments of this study respect the ethical standards in the Helsinki Declaration of 1975, as revised in 2008(5), as well as the national law.

Financial support:
This paper was financed by a grant from the Competitiveness Operational Programme 2014-2020, Tissue engineering technologies for cardiac valve regeneration, valve-regen, id P_37_673, Mysmis code: 103431, contract 50/05.09.2016, NIH grant P30GM131959, NIH grant 1R01HL133303-02 and the Dempsey Endowment.

Conflict of interests:
Leslie Sierad is also an employee of Aptus Bioreactors Company.

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