Title: **Hemodynamic evaluation of Tetralogy of Fallot surgical repair using a soft biorobotic heart, in silico, and ovine models**

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One Sentence Summary: Soft robotic biohybrid heart benchtop and computational fluid models support biomaterial monocusp valve assessment for Tetralogy of Fallot correction.

OVERLINE: BIOENGINEERING

Abstract: Tetralogy of Fallot is a congenital heart disease affecting newborns and involves stenosis of the right ventricular outflow tract (RVOT). Surgical correction often widens the RVOT with a transannular enlargement patch, but this causes issues including pulmonary valve insufficiency and progressive RV failure. A monocusp valve can prevent pulmonary regurgitation, however, valve failure resulting from factors including leaflet design, morphology, and immune response can occur, ultimately resulting in pulmonary insufficiency. A multimodal platform to quantitatively evaluate shape, size, and material and the effects of these variables on clinical outcomes could optimize monocusp design. This study introduces a benchtop soft biorobotic heart model, a computational fluid model of the RVOT, and a monocusp valve made from an entirely biological cell-assembled extracellular matrix (CAM) to tackle the multifaceted issue of monocusp failure. The hydrodynamic and mechanical performance of RVOT repair strategies were assessed in biorobotic and computational platforms. The monocusp valve design was validated in vivo in ovine models through echocardiography, cardiac magnetic resonance, and catheterization. These models supported assessment of surgical feasibility, handling, suturability, and hemodynamic and mechanical monocusp capabilities. The CAM-based monocusp offered a competent pulmonary valve with regurgitation of 4.6 ± 0.9% and transvalvular pressure gradient of 4.3 ± 1.4 mmHg after seven days of implantation in sheep. The biorobotic heart model, in silico analysis, and in vivo RVOT modeling allowed iteration in monocusp design not currently feasible in a clinical environment and will support future surgical testing of biomaterials for complex congenital heart malformations.

INTRODUCTION

The Tetralogy of Fallot (ToF) is a common form of cyanotic congenital heart disease, affecting 7 to 10% of newborns (*1*), with a prevalence of 3.5 cases per 10,000 births (*2*). ToF is characterized by four heart defects occurring together including pulmonary valve (PV) stenosis, right ventricular hypertrophy, a ventricular septal defect, and an overriding aorta. These defects cause a misaligned right ventricular outflow tract (RVOT), impairing effective blood pumping. The extent of RVOT obstruction varies, determining cyanosis severity. Surgical correction involves enlarging the obstructed RVOT, often requiring transannular patch placement by cutting through the PV annulus (*3, 4*). However, this procedure causes PV regurgitation, shifting the right ventricle (RV) from pressure-overloaded to volume-overloaded, leading to arrhythmia, RV dilation, and heart failure (*5*). Valve-sparing procedures assist with pulmonary regurgitation but are unsuitable for severely stenotic cases. Valved conduits made of xenogenic biomaterials offer an alternative but have limited lifespans, often requiring multiple reoperations due to patient outgrowth (*6*). For ineligible valve-sparing cases, monocusp valve insertion is used as a stopgap procedure to address pulmonary regurgitation and volume overload (*7*). Monocusp insertion can decrease stays in the intensive care unit, as well as lower rates of morbidity and mortality (*8*). Nevertheless, cases of progressive pulmonary insufficiency are documented in patients undergoing monocusp reconstruction (*9*).

Potential causes of monocusp dysfunction include leaflet design, morphology, and immunological responses to materials. Monocusp valves are surgically created with variations in design influenced by factors like length, leaflet edge proximity, redundancy for patient growth, and shape (*10-13*). These valves are made from chemically processed animal-derived material, for example, crosslinked bovine pericardium or synthetic polytetrafluoroethylene (ePTFE)

material (*7, 13*). Immune-related failure can lead to immobile valve cusps due to chronic inflammation from foreign materials, causing issues like thromboembolic complications or infectious endocarditis (*14-16*). Post-treatment regurgitation rates vary substantially due to the numerous variables of design, morphology, surgery, and immune response, (*17*). Factors contributing to monocusp dysfunction in the RVOT are not adequately quantified, partly due to limitations in current materials and characterization techniques in monocusp design literature.

Animal studies are frequently used to assess the functionality and mechanical competence of monocusp valves and transannular graft procedures, as well as the suitability of new materials and techniques. However, rodent studies do not reflect the relevant sizes and structures for humans (*18*). Large animal models offer a more suitable platform for evaluation, closely mimicking human hemodynamics and size (*19, 20*); however, cost and ethical concerns limit the number of design parameters that can be assessed (*20*). In vitro mock circulatory flow loop models of the RVOT provide an alternative to animal-based research; however, they oversimplify the anatomical and biomechanical complexities of the RVOT and lack adaptability for surgical modifications (*21-23*). Existing computational RVOT models are static due to the challenge of modeling dynamic valve leaflets. Creating in silico models capable of representing the folding and buckling of delicate leaflet material requires extensive material characterization and computational models not yet available for monocusp designs. A precise experimental or computational fluid model that replicates leaflet dynamics, physiological hemodynamics, and three-dimensional motion of the right heart could greatly benefit the evaluation of new monocusp designs for congenital heart defects (*24, 25*). Employing a benchtop approach could also reduce the need for expensive animal studies, facilitating the investigation of design parameters with improved reliability, accuracy, and control. To address these unmet needs, we

present a soft biorobotic hybrid heart benchtop system that emulates both the anatomical and biomechanical characteristics of the heart. This system is complemented by an advanced computational fluid model of RVOT for the clinical assessment of diverse monocusp types and surgical designs.

We tested the use of a cell-assembled extracellular matrix (CAM) biomaterial to create pulmonary monocusp valves and transannular patches, aiming to mitigate complications associated with foreign materials (*26-28*). CAM, a non-living, allogeneic, and entirely biological graft, is synthesized from human or ovine dermal fibroblasts using tissue engineering techniques. CAM sheets have been successfully used in vascular tissue, serving as both simple sutures and woven grafts (*26, 29-31*). To tackle the multifaceted challenge of monocusp materials and design, we evaluate the surgical viability, hemodynamic efficacy, and mechanical performance of the CAM-based monocusp valve using both ex vivo biorobotic heart and in silico models. Insights from ex vivo and in silico testing optimize the CAM-based monocusp valve design, followed by evaluation in clinically relevant ovine models for hemodynamic performance. Integration of the biorobotic heart, in silico, and in vivo models offered an understanding of the impact of valve placement on flow patterns, providing valuable insights for clinical decisions in RVOT repair surgery, which can be more broadly applied to other surgical approaches.

RESULTS

Longer culture duration enhances the mechanical properties of ovine-derived CAM sheets. We have previously demonstrated our ability to translate CAM sheet production from human or ovine cells (28). Here, we used ovine fibroblasts for allogeneic implantations in sheep (**Fig 1A**).

Before producing CAM sheets, fibroblast cells harvested from the ovine dermis were amplified and frozen down. Frozen cells were thawed to produce the CAM sheets, and the culture was expanded twice (**fig. S1**). The cells were cultured in the presence of ascorbic acid and fetal bovine serum that stimulated extracellular matrix (ECM) secretion and assembly. We evaluated the culture time of ovine fibroblasts for the effect on perforation strength, hydroxyproline content, maximum tensile force, and thickness of the CAM. CAM strength increased linearly with culture time in the perforation assay ($R^2 = 0.92$, **Fig. 1B**). In addition, hydroxyproline quantification, an indicator of collagen content (*32*), demonstrated that the amount of collagen also increased linearly over the culture time ($R^2 = 0.88$, **Fig. 1C**). Furthermore, tensile test (**Fig. 1D**) and thickness (**Fig. 1E**) measurements showed that maximum tensile force and thickness of the CAM linearly increased over the time of culture up to 24 weeks ($R^2 = 0.98$ and $R^2 = 0.94$, respectively). Hydroxyproline content of the CAM correlated with perforation strength and maximum tensile force (Pearson r = 0.99, $R^2 = 0.99$, **fig. S2, A** and **B**). Histological observations confirmed that the collagenous structure of the CAM sheet (blue) became more dense over the time of culture (**Fig. 1F**).

Suture retention strength and uniaxial mechanical properties evaluated in the long-axis direction of CAM sheets increased significantly between 8- and 16- weeks of culture ($P<0.0001$) (**Fig. 1, G** and **H**). The ultimate tensile strength of 8- or 16-week-old CAM sheets was lower than Xenosure patches, which are glutaraldehyde-treated bovine pericardium. CAM materials cultured for 16 weeks exhibited ultimate tensile strength nearly two times higher than literature values of human aortic and PV cusps (*33*) (**Fig. 1H**). The slope of the linear region of the stress-strain curves (**fig. S3**) was used to determine Young's modulus of elasticity (**fig. S4**). CAM sheets cultured for 8- or 16- weeks had lower Young's modulus than Xenosure (**fig. S4**).

For cardiac valve tissue engineering applications, we tested 8- and 16-week-old CAM sheets for the construction of a monocusp valve and transannular patch in biorobotic porcine and ovine hearts (**Fig. 1I**). After measuring the diameter of the pulmonary annulus, the shape of the monocusp and the transannular patch was handcrafted by the surgeon. Using synthetic 6-0 Prolene (polypropylene) sutures and standard surgical instruments, we sutured the CAM sheets to the ventricular portion of the opening to create a monocusp valve and a transannular patch (**Fig. 1I**). The 8-week CAM sheets showed insufficient mechanical resistance to suturing; however, the 16-week-old CAM was handled easily and demonstrated coaptation (complete closing of the leaflets) when tested with water by visual inspection. The 16-week-old scaffold was used for further studies.

A biorobotic hybrid heart can drive an experimental mock circulatory flow loop.

A biorobotic heart was developed through a hybrid approach, combining an organic endocardial scaffold with a synthetic soft robotic myocardium. The organic portion of the scaffold was made of porcine or ovine hearts that were chemically preserved for anatomical accuracy. The endocardial scaffold from the animal heart helped to maintain the complex, non-geometric shape of the RV and the RVOT. A CAM-based monocusp valve and transannular patch were surgically grafted to the RVOT (**Fig. 2A**). The tricuspid valve leaflets were replaced with a 19-mm diameter mechanical tricuspid valve (M-TV) (**Fig. 2B**) by making an incision in the right atrium to ensure the forward flow of blood through the outflow tract during systole. Myocardial tissues from the left ventricular and interventricular septal regions were removed by hand-dissection (**Fig. 2C**) and replaced with a custom soft robotic myocardium (**Fig. 2D**) composed of multiple actuators aligned with the underlying muscle architecture and embedded in a silicone soft

elastomer. This synthetic myocardium was inflated and deflated to mimic the pumping action of the heart and allowed the replication of RV wall motion and circumferential contraction of RVOT. The silicone myocardium housed McKibben-style soft robotic actuators, embedded in a biomimetic fashion, that were designed to recapitulate the contractile function of native myocardium.

Microcomputed tomography (μCT) visualization of the biorobotic right heart revealed the preserved and reconstructed anatomical details, including the actuators integrated into the RV free wall and septum (**Fig. 2E**). The tricuspid valve that was replaced with a mechanical valve, which is visible as a bright object. The heart was connected to a benchtop flow loop that recreated the pulmonary circulation and allowed the adjustment of circuit parameters including preload, vascular compliance, afterload, and vascular resistance (**Fig. 2F**). The synthetic myocardium was powered by pneumatic pressure and cyclically activated using a custom-made electropneumatic control system (*34*) enabling the robotic heart to function as the primary pump, driving fluid flow in the circuit. The robotic right heart generated physiological right heart hydrodynamics (**Fig. 2, G** and **H**) including phasic right ventricular pressures (RVP) of 28/2 mmHg, mean right atrial pressures (RAP) of 4 mmHg, and phasic pulmonary artery (PA) pressures (PAP) of 27/13 mmHg. The PA outflow was over 3 L/min at a heart rate of 60 beats per minute (bpm). A pulmonary regurgitation fraction of 14% was calculated by dividing the backflow volume by the forward flow volume measured by ultrasonic flow probes (**Fig. 2H).** The pressure gradients drove flow across the pulmonic valve (**fig. S5**). These data served as a baseline control biorobotic hybrid heart to mimic the heart without RVOT. The contraction of the biorobotic heart displaced fluid and created pressure differentials across the PV, which resulted in the valve opening and closing during the cardiac cycle. The PV was observed by

echocardiography (**Fig. 2I**) and endoscopic imaging (**Fig. 2J**). During systole, the RV pumped fluid to open the PV, which ejected the fluid into the PA (**Fig. 2J**). The PV closed during diastole and allowed the development of PA diastolic pressures and right ventricular filling (**Fig. 2J**).

The biorobotic hybrid heart models hemodynamics of surgical reconstructive techniques with CAM sheets in RVOT repair.

The organic endocardial scaffold of the biorobotic hybrid heart provided an anatomically accurate model of the RVOT and was used to reconstruct various RVOT repair morphologies using conventional surgical techniques. We considered several surgical scenarios. RVOT obstruction due to a narrowed PA, results in a fixed resistance to pulmonary blood flow. We modeled the application of a peripheral PA patch, which can widen the vessel diameter through an incision in the PA wall to relieve the resistance (**Fig. 3A**, **fig. S6**). Conversely, RVOT caused by a misaligned anterior infundibular septum is dynamic and varies with the contractile state and loading conditions of the RV which can progressively lead to RV hypertrophy. To model surgical repair of this small PV annulus, we tested the application of a transannular patch to enlarge the outflow tract by opening the PA anteriorly across the PV annulus and incising the infundibulum (**Fig. 3B**, **fig. S7**). The incompetent PV that results from this procedure can be repaired by inserting a monocusp valve through the ventriculotomy (incision of the heart ventricle) and the remaining native posterior pulmonary cusps are left intact (**Fig. 3C**, **fig. S8**). Another similar surgical strategy for achieving a functional PV is the augmented leaflet technique, in which the native pulmonary cusp tissue is conserved and extended with a patch sewn onto the edges of the separated or cut leaflet (**Fig. 3D**, **fig. S9**). The transannular outflow

tract patch is used in conjunction with the monocusp or the augmented leaflet technique to form a roof over the reconstructed and enlarged area.

The biorobotic hybrid heart replicated right-sided hemodynamics on the bench and enabled the evaluation of hydrodynamic performance and durability of CAM sheets (**Fig. 3, E** and **F**, **movie S1**). The time-series data tracked changes in hydrodynamics related to different RVOT surgical repair conditions. The data included RAP, RVP, PAP, and PA outflow measurements.

In cases where PA stenosis is addressed through a peripheral PA patch, the right-sided pressures and flow closely matched the baseline case (no RVOT reconstruction), with a systolic RVP of 31 ± 3 mmHg, mean RAP of 4 ± 1 mmHg, systolic PAP of 29 ± 2 mmHg, and PA outflow of 3.2 L/min. However, in cases where the PV is dysfunctional due to an incision through the anterior pulmonary leaflet, such as with a non-valved transannular patch, severe pulmonic regurgitation occurred, resulting in equilibration of PA and RV diastolic pressures and an increase in RAP to 10 mmHg (**Fig. 3, E and F**). The PA outflow also exhibited high backflow, seen as a more negative flow during diastole. Both the regurgitant PA flow and diastolic PA pressures were restored once the PV is repaired with either the monocusp or augmented leaflet techniques, reducing the potential for post-operative volume overload from the RV, which may support favorable clinical outcomes and is consistent with the existing literature (*7, 35*). High-frequency oscillations in PAP measurements resulted from pressure sensors placed near the valve and were caused by the valve closing during diastole (**Fig. 3E**). Echocardiography enabled two-dimensional, dynamic imaging of the repaired RVOT morphology, including the reconstructed PV and transannular patch for each case. An optically clear blood mimic allowed for direct visualization of corrected morphology under physiological pressures and flow through an endoscopic camera (**Fig. 3G**). Clinical indicators, such as PV regurgitation and transvalvular

pressure gradient, an indicator of valve stenosis and dilatation, were used to compare PV performance across different repair conditions using in vitro hydrodynamics. The relative regurgitation index, obtained by dividing the regurgitation fraction of a representative repair case by the baseline, was comparable to the baseline in the case of a peripheral PA patch but increased for a transannular patch without a monocusp valve, indicating no functional valve at the annulus (**Fig. 3H**). The index recovered for the cases of augmented leaflet and monocusp insertion, as expected. The transvalvular pressure gradient was estimated by the peak valvular pressure gradient across the pulmonic valve, which was lower than the baseline in the case of a transannular patch alone due to the equalization of RV and PA pressures (**Fig. 3I**). The transvalvular pressure gradient was comparable to the baseline for the PA patch and monocusp conditions but three times higher for the augmented leaflet technique. However, the pressure gradient was within the clinically defined range of pulmonary stenosis, and the increase could be caused by the delayed opening of the augmented leaflets due to suture lines on the cusp. According to clinical literature, mild, moderate, and severe pulmonary stenosis are defined by pressure gradient ranges of 10-35 mmHg, 36-64 mmHg, and greater than 64 mmHg, respectively (*36*).

Based on preliminary benchtop biorobotic hybrid heart testing of the two surgical techniques evaluated for PV reconstruction following a transannular repair (anterior leaflet augmentation or monocusp), the monocusp technique demonstrated performance comparable to baseline for pressures and flows in the RA, RV, and PA (**Fig 3**, **E** to **I**). As a result, further evaluation using computational and animal models focused exclusively on the monocusp design. The effect of pressure overload on the durability and performance of the CAM-based monocusp valve was evaluated on the benchtop through PA banding. Hydrodynamic measurements showed

an increase in the systolic RVP and phasic PAPs, a decrease in PA outflow, but no change in regurgitation fraction, suggesting that the CAM-based valve was capable of withstanding high-pressure conditions (**Fig. 3J**, **fig. S10**).

We employed fixed actuation (~15 psi) and pressure overload for consistent, controlled comparison in various repair scenarios. This method was promising for refining patient-specific hemodynamics pre- and post-operatively. However, to replicate the compensatory mechanism, which adjusts contraction based on overload changes, a hybrid computational-experimental model would be beneficial (*37*). Our experimental hybrid heart and mock circulatory fluid model was adapted to include adaptive actuation mechanisms (**figs. S11-S13**, **movie S2**). This hybrid computational-experimental benchtop model involved a feedback loop with flow sensors for predicting and responding to overload. For example, to compensate for reduced cardiac output caused by a higher afterload, the native heart may increase its contractility, which helps it pump blood more effectively against the higher resistance. The biorobotic hybrid heart systolic contraction reacted to pressure afterload in the outflow circuit, leading to increased RV chamber pressure and automatic adjustment of actuation pressure in response to changes in outflow resistance and mean pulmonary outflow (**fig. S13**).

The computational fluid dynamics model predicts hemodynamics of surgical RVOT repair. A computational model was developed to simulate four distinct scenarios: native PV, RVOT obstruction, the transannular patch only, and monocusp valve insertion. The biorobotic model was used to create a virtual transannular surgical incision and repair operation for RVOT obstruction geometry **(Fig. 4A)**. Using this virtual surgical repair technique, we provided

physiological models for valve repair scenarios, including transannular patch and monocusp valve models, and replicated the right-sided hemodynamics in silico.

The velocity distribution in both the short-axis plane and the anterior-posterior long-axis plane were modeled, allowing a comparison of transvalvular flow distribution during systole across these scenarios (**Fig. 4B** and **movie S3**). As expected, the maximum velocity was localized to the valve orifice. At peak systole, the axial velocity gradients near the valve orifice were very high, indicating a peak velocity of about 250 cm/s in the case of an obstructed RVOT (note different range on the velocity color bar for this case). The healthy peak velocity across the PV is reported to be 80-120 cm/s (*38*). The streamline pattern analysis revealed a peak velocity jet flow of 100 cm/s at the valve orifice during systole for the monocusp. As anticipated, this observed value is comparatively lower and closer to the native range compared to the RVOT obstruction model.

Quantitative flow across the native PV, RVOT obstruction, and through the valve repair scenarios (monocusp and transannular patch), were predicted by in silico modeling (**fig. S14)**. The native or healthy in silico model demonstrated a benchmark mean flow rate of 158.1 mL/s, falling within the published range of flow values in the literature for sheep (*39*). In comparison to a mean flow of 67.1 mL/s for the transannular patch only, the monocusp valve could restore the fluid flow and represented a mean flow of 119.5mL/s under equal pressure conditions as the healthy state. The velocity contours and flow during diastole (**fig. S15)**, showed that only the transannular patch hinders the optimal functionality of the valve, resulting in retrograde flow due to insufficient valve closure. The inclusion of a monocusp valve to a transannular patch was beneficial in minimizing reverse flow in the in silico model, hence facilitating the functional valve closure comparable with the healthy state (**fig. S14** and **S15**).

CAM-based monocusp and transannular patch demonstrate in vivo hemodynamic and mechanical integrity in an in vivo ovine model.

To evaluate the in vivo functionality, mechanical behavior, and hemodynamic performance of the CAM sheet, we implanted custom monocusp valves and transannular patches in three adult sheep using standard surgical instruments and procedures. Hemodynamic data were collected through cardiac catheterization, echocardiography, and magnetic resonance imaging (MRI). All sheep underwent baseline measurement and valve and patch implantation. Initially, baseline values for catheterization and echocardiography (no MRI) were recorded in all sheep to serve as a control dataset for comparison. The same sheep then underwent RVOT repair, which included both a monocusp valve and a transannular patch, to evaluate the effectiveness of the combined intervention in vivo (**fig. S16)**. We did not conduct the "transannular patch only" surgical scenario due to potential complications in a survival study with an incompetent PV. All animals survived the procedures without complications.

At the beginning of the study, right-heart pressures were measured both before (day 0 pre-implantation) and after (day 0 post-implantation) monocusp implantation using RV catheterization. Echocardiography was used to qualitatively assess PV motion and leaflet morphology before and after implantation. Right ventricular anatomy and PV flow were evaluated by MRI on day 7. A terminal procedure was performed on day 7 post-implantation, which included RV catheterization and epicardial echocardiography. Images taken during and after the surgical procedure, as well as on day 7, highlighted the reconstructed RVOT with a CAM-based monocusp and transannular patch (**Fig. 5A**). Immediately upon the restoration of blood flow on day 0, a bulging of the transannular patch was observed and remained constant for

seven days, showing no signs of further dilation (**Fig. 5A**). The CAM is thinner than the vessel wall (at 270 μm), and is oversized, leading to this bulging. The modulus (35 MPa) is higher than the vessel wall which may lead to stress transfer, decreasing the circumferential tension within the artery wall.

Representative pressure waveforms from RV catheterization demonstrated sustained pressure differences between the RV and PA and indicated that the reconstructed CAM-based monocusp valve was functioning well under physiological pressures both on Day 0 after implantation and on Day 7 **(Fig. 5B)**. Clinical metrics were collected for diagnostic assessment of the monocusp PV, including transvalvular pressure gradient (<6 mmHg), pulmonary regurgitation (4.6 ± 0.9 %), cardiac output (3.4 ± 0.7 L/min), and ejection fraction (39.7 ± 3.8 %) **(Fig. 5, C and D).** Epicardial echocardiography showed the morphology of monocusp valves and confirmed that the CAM-based monocusp valve remained pliable and functional after undergoing physiological stresses for seven days (**Fig. 5E**, **movie S4**). The monocusp had a longer length than the native leaflet (double-sided white arrows, **Fig 5E)**. Monocusp valves are typically designed with additional material to accommodate growth, ensuring sustained functionality as the patient grows.

Pressure waveforms (RVP and PAP) for a monocusp valve across a cardiac cycle were similar in both ex vivo (simulated) and in vivo (physiological) studies (**Fig. 6A**). Peak-to-peak differential pressure (dP) across the PV between native and monocusp valves were similar for simulated and physiological conditions (**Fig. 6B**). The findings suggested the promise of the CAM-based monocusp technique in restoring PV function for patients who require RVOT reconstruction and supported the suitability of the benchtop model for evaluating cardiac interventions.

Hemodynamics of the biorobotic heart, in silico, and in vivo models were similar after RVOT reconstruction using a CAM sheet.

During diastole, blood from the PA filled the monocusp, pushing it against the remaining native PV leaflets, and thereby created the coaptation zone. During systole, the monocusp opened, allowing blood to flow through when the monocusp was flush with the anterior wall of the RVOT and the CAM outflow tract patch. The function and the opening and closing motion of the CAM-based monocusp was evaluated in vivo on day 7 using MRI (**movie S5**). The standard velocity-averaging method used in the MRI assessment showed no pulmonary insufficiency in any of the animals following monocusp implantation. The comparison between flow waveforms obtained from 2D-PC MRI data and computational models suggested agreement and supported the in silico model (**Fig. 6C**). The flow analysis at the monocusp valve revealed a peak systolic flow rate of 245.6 ± 7.7 mL/s for an average cardiac cycle in vivo, compared to a peak flow rate of 237 mL/s in silico. The regurgitant volume for the in silico control, consisting of a transannular patch only, was predicted to be 35.6 mL, which was reduced to 2.2 mL, 3.4 mL, and 2.1 ± 1.3 mL after CAM-based monocusp implantation for ex vivo, in silico, and in vivo experiments, respectively (**Fig. 6D**, **fig. S17**, **fig. S18**).

CAM material integrates with native tissue with minimal early inflammatory reaction after seven days of implantation as a monocusp valve and transannular patch.

A segment of the RVOT was harvested after the terminal study on day seven, fixed using formaldehyde, and qualitatively assessed to show the intact monocusp valve, native preserved leaflets, and transannular patch (**Fig. 7A**). The RVOT was also opened longitudinally to better

visualize the monocusp and the two native pulmonary leaflets (**Fig. 7A**). Explanted tissue cross-sections were evaluated by hematoxylin-eosin-saffron staining to ascertain if there was any recellularization (**Fig. 7B**). As expected, at this short-term time point (7 days), we observed a thin layer of fibrin (visible in pink) containing blood cells that was deposited on the CAM surface in blood contact. Immunostaining allowed the identification of cells that co-express alpha-smooth muscle actin (alpha-SMA) and calponin within the fibrin layer (**Fig. 7C**; left panel). These cells may be smooth muscle progenitor cells that came from the blood circulation (*40*). As expected, we also identified the presence of lymphocytes ($CD45^+$) and T-lymphocytes ($CD3^+$) in the fibrin layer (**Fig. 7C**; right panel). Although some cells were observed within the first few microns of the monocusp and the transannular patches, we did not observe cell infiltration in the middle of the CAM material nor at its junction with the native tissues. However, observations at the interface between the monocusp root and the myocardium, and the approximation between the transannular patch and the PA showed good integration of the CAM material (**Fig. 7B**, dotted blue inset). Furthermore, a small number of pro-inflammatory type 1 macrophages ($CD64^+/CCR7^+$) were observed at the interface of the CAM material and the native tissue (**Fig. 7D**, **fig. S19**). Longer-term in vivo studies are needed to further evaluate the immune response and tissue remodeling.

DISCUSSION

In this study, we demonstrated the effectiveness of soft robotic benchtop and computational biomechanical models for evaluating the hydrodynamic and mechanical performance of CAM material as a monocusp valve, intended for patients with RVOT stenosis in ToF. There are several advantages to these models: First, the monocusp leaflets and other RVOT repair types

can be surgically placed into our isolated beating RVOT model on a bench using standard surgical techniques to closely reproduce the true implantation conditions, with lower costs and ethical considerations compared to use of many animals. Second, the biorobotic heart model can assist in replicating clinically representative hemodynamics by recapitulating the flow and pressure conditions after different simulated surgical repairs, enabling direct performance comparison and contrast. The fully coupled fluid-structure interaction computational model with accurate material characterization allows the simulation of in silico surgery and can predict parameters such as the change in regurgitant volume in the pre- and post-RVOT repair states. The proposed computational model is applicable for an in silico patient-specific pre-surgical planning framework for RVOT repair, patch reconstruction, and monocusp valve shape optimization. By applying the computational framework to pre-surgical imaging data, we generated a 3D reconstruction of patient-specific RVOT/PA and provided a mechanism to predict the best post-operative patch and valve design features in the future.

This system can also potentially explore the impact of various designs of monocusp valves on clinical outcomes. Furthermore, these platforms can be used with different valve materials, including biologic homograft patches for example, autologous pericardium (*42*), and could be adapted to study other geometrically complex RVOT interventions, such as the design of RV to PA conduits (*43*). Though the in silico surgical geometry in the presented work assumed a zero initial stress and strain state, the proposed computational framework could include an inverse step solution to calculate the actual stress state caused by the incision. This would allow capturing tissue wall motion, such as retraction due to residual stress, resulting in more realistic surgical planning for optimal design features of the monocusp valve. Overall, the presented

approaches can provide a more quantitative evaluation of the mechanical effectiveness and hemodynamic performance of new graft materials, ultimately reducing the need for expensive and invasive animal studies. Developing such platforms could be a crucial step in improving and understanding the long-term clinical outcomes of patients with congenital heart defects.

The introduction of an allogeneic CAM-based monocusp valve and transannular patch for correcting ToF widens the range of available biomaterials for cardiovascular interventions. We evaluated the hemodynamic and mechanical performance of the CAM-based monocusp valve using the biorobotic and computational models, as well as in vivo ovine animal models through RV catheterization and clinical imaging tools. The findings indicate that after seven days of implantation, the monocusp valve acted as a competent PV with a low regurgitation fraction and a low transvalvular pressure gradient. Consistent with our previous findings (*44*), we confirmed that CAM parameters such as the perforation strength, the tensile strength, the hydroxyproline content, and the thickness linearly increase with the time of culture, suggesting that we can adapt the material to a desired clinical application. Prior research has confirmed the successful implantation of CAM-based tubular vascular grafts in humans (*27, 28, 45-48*). CAM produced by allogeneic fibroblasts caused no adverse immune response (*45*) and was devitalized for long-term storage without loss of structural integrity (*29*). Further, devitalized human CAM materials do not trigger a degradative immune response for up to six months, but rather induce a very slow remodeling (*49*), making CAM a potential candidate for monocusp construction while mitigating immunological responses from synthetic materials which could potentially address the limitations of current materials for ToF repair. Although current materials improve the survival and quality of life, they are not permanent solutions, especially for pediatric applications, and

entirely biological, human, and non-chemically modified tissue such as the CAM material could potentially represent an advance for the treatment of the pediatric population.

CAM-based tissue-engineered constructs have previously demonstrated successful integration with native tissue and resistance to infection and remodeling by host cells (*27, 48, 50*). The growth potential of pediatric tri-tube valved conduits made from fibroblast-produced ECM was previously demonstrated (*51*) using a method that allowed cells to grow in a tube made of fibrin gel (*52*), which was subsequently decellularized (*53*). This biological material integrated and grew in lambs for 52 weeks as a valved conduit with reduced calcification and improved hemodynamic function. Based on these findings (*51*), we posit that our CAM-based model could potentially be populated with the patient's own cells and have the potential for growth and thus provide an alternative option for patients with congenital heart diseases, such as ToF, who currently require multiple surgeries to accommodate growth and address complications associated with synthetic and xenogeneic patches (*54*).

Several limitations of the study are acknowledged. First, the stiffening of heart tissues in the biorobotic model due to formalin fixation could potentially be addressed by surfactant washing, that has been previously demonstrated to enhance mechanical properties (*55-57*). We have not yet explored how long-term cyclical loads affect the mechanical properties of the CAM material. Extended studies are necessary to evaluate the durability and the effect of any in vivo remodeling. Although we did not see a structural modification of the CAM-based transannular patch after 7 days, future work will include analysis of explanted leaflets to assess mechanical and structural changes over time. The observed positive pressure differences during early diastole in **fig. S5** were primarily due to highly oscillatory pressure responses, recorded by the sensors in the afterload circuit of the mock flow loop. These fluctuations resulted from the rapid

expansion and recoil of materials, such as latex tubing due to fluid movement within the flow loop. In some instances, there was a brief period where blood continued to flow after the coaptation of the monocusp valve, possibly due to the closing dynamics of the valve, the momentum of the blood and inertial effects.

In conclusion, this study described the use of an allogeneic CAM-based monocusp valve and transannular patch, as well as models for their hemodynamic characterization. This approach has the potential to advance the materials and characterization tools available for congenital heart defects and could ultimately improve the outcomes for patients in need of reconstructive surgeries. Beyond the evaluation of a monocusp procedure for ToF, the model platforms described here could be more broadly applied to study surgical and minimally invasive interventions and their effects on hemodynamics.

MATERIALS AND METHODS

Study design

The goals of the study were to develop models to evaluate surgical correction of Tetralogy of Fallot and to test various surgical approaches with a cellular-derived biomaterial. We created a candidate biomaterial monocusp using allogeneic fibroblasts and first evaluated the properties and performance of these CAM sheets in vitro and ex vivo. The biomaterial was characterized in vitro for the ability to resist suturing and surgical manipulation by assessment of perforation, hydroxyproline content, tensile properties, thickness, and suture retention. The biomaterial monocusp CAM sheets were then tested in explanted animal hearts using our benchtop soft biorobotic beating heart with preserved ovine or porcine tissue. The hemodynamic performance

of different surgical repair strategies of the RVOT was tested in the biorobotic heart, which mimicked the cardiac function of the right side of the heart. An in silico RVOT model was used to further study the fluid dynamics of the monocusp valve, predicting flow characteristics and valvular velocities for simulated surgical repairs (**fig. S20**). Based on in vitro and ex vivo results, one condition was tested in vivo in three sheep in a non-randomized manner. The mechanical stability and functionality of the CAM pulmonary monocusp valves and transannular patches were assessed through seven days of ovine implantation by MRI analysis, echography imaging, pressure measurements, and histological analyses. The surgical team and investigators were not blinded during analyses. All animal procedures in this preclinical study were conducted in compliance with the National Society for Medical Research Principles of Laboratory Animal Care and aligned with the EU Directive 2010/63/EU. The study was carried out in accredited animal facilities (IHU Liryc, accreditation #A333183), and it was approved by the Animal Research Committee (protocol authorization: APAFIS#36013-2022031816469503).

Fabrication of CAM sheets

Ovine fibroblasts were harvested from a post-mortem skin biopsy (approximately 2 cm^2) of a one-year-old female sheep (**fig. S1**). The cells were isolated using an explant culture process, as previously described (*28*). Briefly, the dermis was cut into small fragments (1 to 2 mm^2) under sterile conditions and placed in T25 culture flasks. Primary culture (passage 0, P0) of the fibroblasts was established for cellular expansion in fibroblast medium (Dulbecco-Vogt modified Eagle medium with Ham's F12 nutrient mixture at 3:1 with 2.6 mM glutamine; HyClone Laboratories -GE Healthcare Life Sciences) supplemented with 20% Hyclone fetal bovine serum III (HyClone Laboratories) and 1X Penicillin-Streptomycin (Gibco). The culture was maintained

in a humidified incubator at 37°C with 5% CO_2. The fibroblast cells were then subcultured one to six times (P1 to P5) at a density of $1x10^3$ to $2x10^4$ cells/cm^2 and cryopreserved (**fig. S1**). To obtain CAM sheets, ovine fibroblasts were culture expanded two times for one week each at a density of $1x10^4$ cells/cm^2 (P6 to P7) in T-125 flasks (Falcon). The cells were then seeded at a density of $1x10^4$ cells/cm^2 (P8) in T-225 flasks (Falcon) and cultured for 16 weeks in DMEM/F-12 supplemented with 10% Hyclone fetal bovine serum III and 500 μM sodium ascorbate (Sigma-Aldrich). Four-, 8-, 16-, and 24-week-old CAM sheets were also produced for suture retention and tensile tests. The medium was changed three times per week. At the end of the culture, CAM sheets were washed with sterile MiliQ water and stored at -80°C until use.

Fabrication of biorobotic hybrid heart

Mammalian hearts (porcine or ovine) were fixed for three days using a 10% formalin solution and rinsed with 1 M phosphate-buffered saline (PBS). Ovine hearts were used in all cases of RVOT repair, and porcine hearts, matched approximately in size and weight to the ovine hearts, were utilized to establish baseline hemodynamics. The surgically corrected hearts were converted into biorobotic hybrid hearts following previously published methods (*55, 57-60*). Briefly, the endocardial structures were preserved in each heart, and the native myocardium was partially removed (*61*) and replaced with a synthetic soft robotic-based myocardium designed specifically for that heart. The artificial myocardium consists of pneumatic artificial muscles (*34*) strategically placed across the right ventricular free wall and intraventricular septum to mimic the physiological wall motion of the right side of the heart. About 6-8 artificial muscles were secured and positioned relative to each other using a thin (5 mm) elastomeric silicone matrix (Ecoflex 00-35 Fast), resulting in a soft robotic myocardium tailored to each heart's unique

endocardial scaffold. The number and orientation of the artificial muscles were previously optimized through computational modeling (*62*). To ensure the competency of the tricuspid valve, a 19-mm-diameter mechanical valve (St. Jude Medical) was used to replace the native tricuspid valve through a right atrial atriotomy.

Surgical techniques for PV correction

To surgically implant the monocusp valve, a 4 cm long transannular incision was made along the RVOT of each fixed heart, 2 cm long along the infundibulum, and 2 cm long along the main PA, with a divided annulus. The cut native leaflet was then removed, and a handcrafted piece of CAM sheet (approximately 2 cm by 2 cm) was cut sutured at the endocardial side of the heart, spanning from the bottom area of the cut infundibulum to the top of the native leaflet commissure, using 6-0 Prolene (polypropylene) sutures (Ethicon). The incision was closed with a CAM-based transannular enlargement patch sutured at the main PA, pulmonary annulus, and the epicardial side of the infundibulum.

The augmented leaflet technique followed a similar surgical procedure, except that the damaged native leaflet was preserved and used to suture the CAM-based valve. The CAM patch was sutured to each cut cusp edge, leaving 2 mm protruding over the free edge of the valve. For the peripheral PA patch model, a 2 cm long incision was made approximately half cm above the PV annulus and closed with a CAM-based patch for enlargement to recreate a clinically relevant repair scenario.

Mock circulatory flow loop

To simulate the right heart circulation, a mock circulatory loop consisting of hydraulic and mechanical components was used. In-house-built acrylic compliance chambers represented the venous and pulmonary compliances, and on-off ball valves from McMaster-Carr were used to simulate the pulmonary arterial and pulmonic vascular resistance. The biorobotic hybrid heart was connected to the circuit and actuated at 60 bpm using a custom-made electro-pneumatic control box to mimic the pumping function of the cardiac muscle (*34, 63*). The degree of actuation, resistance, and compliance were adjusted to achieve the desired right-sided hydrodynamics. A blood mimic fluid consisting of 40 v/v % propylene glycol in deionized water with a dynamic viscosity of 4.3 ± 0.8 mPa s was used. Hemodynamic parameters were measured using pressure sensors (PRESS-S-000, PendoTECH). Data was recorded using PowerLab and LabChart Pro v8.1.16 software (AD Instruments). Pressure sensors were placed at the right atrium, RV, and the PA by 3.5 F umbilical vessel catheters (CardinalHealth) to measure the biphasic pressure waveforms. The ultrasonic flow probe (ME 13 PXN, Transonic), connected to a T420 multichannel research console (Transonic Systems), was mounted directly onto the PA to record the outflow downstream to the valve. To visualize the valve motion, an endoscopic camera (1080P HD, NIDAGE) was used to record videos at 30 fps. The data were analyzed and plotted with OriginPro 2021b software.

Computational model

Fluid-structure interaction analysis (FSI) was conducted to simulate the motion and function of valves in a three-dimensional RVOT geometry. Three-dimensional RVOT geometry was obtained from an anatomic model of the human heart created from computed tomography and magnetic resonance images (Living Heart Model, Simulia, Dassault Systèmes). The three-

dimensional RVOT geometry was modified using solid modeling computer-aided design (CAD) software (Meshmixer 3.5, Autodesk; SolidWorks 2021, Dassault Systèmes) and imported into Abaqus 2022 software (Simulia, Dassault Systèmes). We created the RVOT obstruction model by scaling down the original model and fixing the annulus area to 3 cm^2, based on planimetry. The three-dimensional RVOT model was then enlarged to reach an annulus area of 5.1 cm^2 to make transannular patch and monocusp valve models. The virtual transannular surgical incision was produced by combining ellipsoidal geometry and RVOT geometry using boolean operations. The final CAD geometry was imported into the finite element solver, and the resulting elements from the meshed geometry represented the stress- and strain-free state. The monocusp leaflet was defined as the geometric profile created by the ellipsoidal boolean cut. The ellipse's long axis length was defined as the same as the incision height, and the ellipse profile had a minor axis length the same as the transannular patch width. The height of the monocusp leaflet was equivalent to the semi-long axis length of the ellipse profile. Nonlinear explicit dynamic analysis was performed to simulate the mechanical response of the FSI analysis. For this study, the modified anisotropic hyperelastic Holzapfel–Gasser–Ogden material model (*64*) was adopted to characterize the mechanical behavior of native PV leaflets. Local coordinate systems were defined for each leaflet to include local fiber orientation. We assumed that the mean fiber directions were symmetric with respect to the circumferential axis of the local coordinate system. The particular material used for native leaflets is the human pericardium, and the constants were previously described for the native human pericardium (*65*). Homogeneous human arterial wall properties were assigned to the artery by using the third-order Ogden isotropic hyperelastic model (*66*). This model captures the averaged behavior of the three layers of the artery: intima, media, and adventitia (*66*). To simulate the obstruction of the RVOT, PV stenosis was created by

partially fusing the commissures of the tricuspid PV. The valve stenosis was assumed to be homogeneous, and the material qualities of the native human pericardium remained unchanged, comparable to its healthy state. The isotropic linear elastic model was employed to characterize the mechanical properties of the CAM sheet for the monocusp valve. The material parameters of the CAM sheet were determined by uniaxial tensile testing, as shown in **Fig. 1.** The **table S1** summarizes the parameters used (*66, 67*). Four-node tetrahedral element (C3D4) was used to simulate the mechanical response of native leaflets and PA, and a 4-node, quadrilateral, stress/displacement shell element (S4) was used to simulate the monocusp valve and transannular patch.

A 2-way coupled FSI modeling technique was adapted to compute intravascular hemodynamic interactions with highly deformable valve structures. An iterative explicit 2-way coupling approach was employed to solve the numerical FSI problems. Commercial structural explicit finite element solver (Abaqus/Explicit, Dassault Systèmes) was coupled with a commercial fluid solver package (XFlow 2022x, Dassault Systèmes) using Abaqus Co-simulation Engine (CSE). The fluid domain was spatially discretized, and governing equations for both domains (fluid and structure) were solved for each discrete time. The fluid-structure system was modeled using the immersed finite element or finite difference method which helped to prevent problems with mesh motion and regeneration caused by the large deformations (*68*). The applied numerical strategy incorporates an information exchange procedure between the finite element solver and the fluid solver at each time step, and solid-solid contact is directly handled within the finite element solver during the co-simulation. Contact between the leaflet structures was modeled by the solver Abaqus using a penalty-based general-contact algorithm which searches for node-into-face and

edge-into-edge penetrations in its current position (*69*). The flow patterns within the RVOT are simulated using a Large-Eddy Simulation (LES) turbulence model.

The blood was represented mathematically as an incompressible Newtonian fluid with a density of 1050 kg/m^3 and a dynamic viscosity of 0.0035 Pa.s. The inlet and outlet boundary conditions for this analysis were derived from measured RVP and PAP pressure data obtained from in vivo studies. Two cardiac cycles at 125 bpm were simulated, and the findings from the subsequent cycle were evaluated, considering that the variation in monitored physical quantities was less than 5% between the two cycles. The stable time increment was set to 1×10^{-5} s to ensure numerical stability and temporal accuracy. Grid independence was tested on the RVOT obstruction case model using three different grid sizes (0.8 mm, 0.6 mm, and 0.4 mm lattice resolution). The convergence of the stability parameter was obtained with a maximum 2.89% difference by checking pressure, velocity, and numerical viscosity between the medium and fine grids; hence, the medium grid (0.6 mm resolution, 205,000 elements) was assumed to be grid-independent. Each FSI simulation was completed in approximately 19.5 hours on a desktop PC with a 3.0 GHZ i7-9700 processor with 8 cores and 32 GB RAM.

Animal preparation and surgery

Before implantation, sterile CAM sheets were thawed at room temperature for 10 min and then rehydrated using a sterile heparin solution (50 UI/mL in saline solution) for 72 hours at 4°C. Allogeneic CAM sheets, produced from ovine fibroblast cells, were grafted as a monocusp valve and a transannular patch in one-year-old female sheep for a period of seven days (n = 3 animals). The animals were premedicated with an intramuscular injection of ketamine (10-20 mg/kg), acepromazine (0.1 mg/kg), and buprenorphine (10 µg/kg/day) before surgery. An intravenous

propofol bolus injection (1 mg/kg) was used to anesthetize the animals and they were maintained on 1.5-3% isoflurane during surgery. A left thoracotomy (instead of a sternotomy) was performed on each animal to facilitate their recovery. After opening the pericardium, a bolus of heparin (3 mg/kg) was intravenously injected. Five minutes later, extracorporeal circulation (ECC) was connected to the heart at a systemic flow of 3 L/m^2/min, with the auxiliary pump flow rate adapted to maintain a pressure of 70 mmHg in the aortic root. The RVOT reconstruction was performed on a beating heart using CAM sheets. As described in the previous section, a 4-cm-long transannular incision was made along the RVOT. The damaged native leaflet was removed, and a piece of CAM sheet was sutured at the endocardial edge of the heart using 6-0 Prolene sutures (Ethicon) to form the monocusp valve. The incision was closed and covered with a CAM-based transannular patch from the distal main pulmonary to the proximal RVOT incision. After ten minutes of observation, air from the right heart chambers was purged, and progressive weaning of ECC was initiated. Hemostasis was controlled, and ECC was disconnected. Heparin was neutralized with an injection of protamine sulfate solution (4.2 mg/kg). A suction redon drain was placed for up to 24-48 hours after the animal woke up, and the thoracotomy was closed and disinfected with silver spray. On the day of the surgery, each animal received antibiotic treatment with amoxicillin (1 mL/10 kg/48 h) until the end of the study. Buprenorphine (10 µg/kg) was administered as a daily analgesic treatment for two days post-operatively. An anti-inflammatory protocol, including intramuscular injection of Flunixin (2 mg/kg) was used during the surgery. To maintain anticoagulation, animals received twice daily subcutaneous injections of enoxaparin (3 mg/kg) and twice daily oral administrations of a clopidogrel pill (375 mg) beginning immediately after the surgery and continuing until the end of the study.

Hemodynamics in vivo

Hemodynamic data were measured in vivo under sterile conditions on anesthetized animals after the thoracotomy both before (baseline) and after RVOT reconstruction (day 0 post-implantation), using catheters connected to pressure sensors. To measure the biphasic pressure waveforms, catheters were placed in the RV and the PA. We also recorded terminal hemodynamics after seven days of implantation. The data were collected using PowerLab (AD Instruments) and recorded with LabChart Pro v8.1.16 software (AD Instruments). The data were analyzed and plotted with OriginPro 2021b software.

Statistical analyses

Data are expressed as the mean ± standard deviation (SD). Statistical analyses were performed, and data was plotted using GraphPad Prism software version 8 (GraphPad Software) or OriginPro 2021b software. All data were tested for normality using Shapiro–Wilk tests (alpha = 0.05) and linear QQ plots. Correlations were assessed by computing two-tailed Pearson correlation coefficients. Differences between two groups were determined using a two-tailed unpaired Student's *t*-test. Otherwise, differences between more than two groups were determined using a one-way analysis of variance (ANOVA) with Bonferroni's multiple comparison test. Differences with $P < 0.05$ were considered significant. Sample sizes are presented in the figure legends. All data are available in **data file S1**.

List of Supplementary Materials

Materials and Methods

Figs. S1 to S20

Table S1

Movies S1 to S5

Data file S1

MDAR reproducibility checklist

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Author contributions:

M.S., F.R., N.L.H., E.T.R., and F.K. conceptualized and designed the study. M.S., F.K., F.R., C.O., P.B., A.R., J.B., S.D.G., S.X.W., J.N., D.E.H., N.D.S., I.G., and C.G. developed the

methodology for data collection and performed experiments. Specifically, A.R., P.B., C.G., and F.K. conducted cell culture work and developed the CAM sheets, A.R., P.B., and F.K. performed mechanical testing and primary histological colorations, A.R. conducted hydroxyproline quantification, F.R., P.B., and F.K. performed ex vivo CAM sheet implantations. M.S. developed the soft biorobotic heart model, J.B. and S.X.W. replaced the tricuspid valve, M.S., F.K., and J.B. performed benchtop echocardiography, C.O. and M.S. designed the computational modeling, F.R. and P.B. performed in vivo grafting of the CAM materials, F.K. performed in vivo echocardiography, F.K., F.R., P.B., A.R. conducted in vivo pressure records, D.E.H. and J.N. performed in vivo IRM imaging, A.R., F.K., and C.O. analyzed the in vivo MRI data, S.D.G. developed the control system to mimic adaptive physiology on bench for the soft biorobotic heart model, N.D.S and I.G. performed terminal histological colorations and immunostainings, and F.K. performed microscope and confocal imaging. Funding was secured by E.T.R., F.K., N.L.H., and F.R. Throughout the process, M.S., E.T.R., N.L.H., F.K., and F.R. supervised the team, maintaining project focus and direction. The manuscript's original draft was written by M.S., F.K., and C.O. M.S., C.O., F.R., F.K., N.L.H., and E.T.R. reviewed and edited the manuscript.

Competing interests: E.T.R. holds a position on the board of directors for Affluent Medical and is also an advisor on the board for Pumpinheart and Helios Cardio. E.T.R. is an academic co-founder of Spheric Bio and Fada Medical. E.T.R. is the inventor of the “Organosynthetic dynamic heart model,” U.S. Patent Application No. 17/193,910. The other authors declare that they have no competing interests.

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Figure Legends

Fig. 1. **CAM culture time affects mechanical properties and suture retention**. (**A**) Image of a 16-week-old cell-assembled extracellular matrix (CAM) sheet. (**B to D**) Graphs showing the linear increase of CAM sheet properties over a 24-week period of culture for mechanical perforation strength ($R^2 = 0.92$) (**B**), hydroxyproline content ($R^2 = 0.88$) (**C**), maximum tensile force ($R^2 = 0.98$) (**D**), and thickness ($R^2 = 0.94$) (**E**). Linear regression is presented as dashed red lines. For (B), 4 weeks, n = 12; 8 weeks, n = 11; 16 weeks, n = 15; 24 weeks, n = 19. For (C), 4, 8, and 16 weeks, n = 10; 24 weeks, n = 8. For (D), 4, 8, and 16 weeks, n = 10; 24 weeks, n = 9. For (E), n = 10 per time point. (**F**) Masson's Trichrome staining for collagen (blue) of 8- and 16-week-old CAM sheet cross-sections. Scale, 50 μm. (**G**) Suture retention strength (N) of the CAM sheets after 8 or 16 weeks of culture. Two-tailed unpaired Student's t-test, **** $P < 0.0001$, n = 16 per time point. (**H**) Ultimate tensile strength (MPa) of CAM sheets cultured for 8 or 16 weeks and clinically available Xenosure biological patches. One-way ANOVA with Bonferroni's multiple comparisons test, * $P < 0.05$; **** $P < 0.0001$; n = 10 per group). Blue and red dotted lines, literature values for mean circumferential ultimate tensile strengths of human PV cusps and human aortic valve cusps, respectively (*33*). (**I**) Images of an explanted porcine heart with a 16-week-old CAM sheet sutured as a monocusp valve. (J) CAM sheet sutured as, a transannular patch. (K) Image of the reconstructed PV from the top showing the native leaflets, and CAM-based monocusp and transannular patch. RV, right ventricle; PA, pulmonary artery. For all panels, data are presented as mean ± SD.

Fig. 2. The **biorobotic hybrid heart supports benchtop evaluation of RVOT repair hemodynamics**. (**A**) Image of a preserved ovine heart with reconstructed RVOT by the insertion

of a monocusp PV and a transannular patch made of CAM sheets. RV, right ventricle; LV, left ventricle; PA, pulmonary artery. (**B**) A preserved ovine heart with insertion of a mechanical tricuspid valve (M-TV). RA, right atrium. (**C**) Image of ovine heart after hand-dissection of thick myocardial tissues from the left ventricular and interventricular septal. (**D**) Image of the removed myocardium replaced with a soft-robotic myocardium to create the biorobotic hybrid heart. (**E**) Microcomputed tomography (μCT) of the grafted biorobotic hybrid heart in the short and long axes after M-TV implantation, RVOT modification, and the soft-robotic myocardium (outlined with red dashed lines). Scale bar, 20 mm. (**F**) Image of the mock circulatory loop for simulating blood flow. (**G**) Pressure waveforms representative of the right side of the heart generated using the biorobotic hybrid heart at baseline without any surgical correction of the RVOT (n=2 porcine hearts, three rounds of testing, 30 consecutive cycles each). RVP, right ventricular pressure (yellow); RAP, right atrial pressure (gray); PAP, pulmonary artery pressure (blue). (**H**) Representative PA outflow waveform recreated in the baseline biorobotic hybrid heart with native PV has not undergone any RVOT correction. Blue shading, forward flow; yellow shading, backflow. (**I**) The native porcine PV visualized through echocardiography in the short and long axis. (**J**) Endoscopic image of the PV leaflets during systole (open) and diastole (closed).

Fig. 3. **Hydrodynamic evaluation of RVOT repair surgical techniques using CAM sheets**. (**A**) Schematic of a heart illustrating a peripheral pulmonary artery (PA) patch to relieve stenosis in the main PA trunk (left). Illustration of transannular repairs made through an incision from the PA to the infundibular muscles of the right ventricle (RV) across the pulmonary valve (PV) annulus (right). (**B to D**) Illustration of the cut native PV leaflet (**B**) before adding transannular patch to widen the RVOT, or (**C**) after monocusp valve, or (**D**) augmented leaflet surgical repair

technique. (**E**) Representative pressure waveform recordings over time in the biorobotic hybrid heart for the case of a peripheral PA patch (*n*=1 heart, three rounds of testing, 30 consecutive cycles each), a non-valve transannular (TA) patch (*n*=1 heart, three rounds of testing, 30 consecutive cycles each), a monocusp valve (*n*=2 heart, three rounds of testing, 30 consecutive cycles each), and an augmented leaflet technique (*n*=1 heart, three rounds of testing, 30 consecutive cycles each). RVP, right ventricular pressure, yellow; RAP, right atrial pressure, gray; PAP, pulmonary artery pressure, blue. (**F**) PA outflow recordings of each RVOT surgical repair case. (**G**) Echocardiography (left) and endoscopic camera images (right) of the morphology and motion of the modified pulmonary valves in the biorobotic hybrid heart. P, peripheral PA patch; A, anterior native leaflet; R, right native leaflet; L, left native leaflet; TA, transannular patch; M, monocusp; AL, augmented leaflet. (**H**) Relative regurgitation index for performance comparison of various RVOT repair cases. (**I**) Valvular pressure gradient as a measure of valvular stenosis. (**J**) The function of the CAM-based monocusp valve under RV pressure overloading. sRVP, systolic right ventricular pressure; sPAP, systolic pulmonary artery pressure; CO, cardiac output. Illustrations created with Adobe illustrator. Images in panel Fig. 3, A are adapted from images by Servier Medical Art (https://smart.servier.com/) under a CC BY 4.0 license https://creativecommons.org/licenses/by/4.0/. I~~llustrations~~mages in panels Fig. 3B, C, and D are created with Adobe Illustrator.

Fig. 4. **Computational fluid dynamics model simulates RVOT repair scenarios and corresponding hemodynamics**. (**A**) Renderings of the in silico model for simulated transannular repair and RVOT reconstruction procedures. PA, pulmonary artery; RV, right ventricle. (**B**) The velocity field distribution in the short- (top) and long-axis (bottom) planes during systole,

simulating native baseline PV, RVOT obstruction, transannular patch only, and monocusp valve insertion. Color scale, velocity, 0-110 cm/s; RVOT obstruction: 0-250cm/s. Renderings created with Solidworks and Meshmixer software.

Fig. 5. **In vivo hemodynamic evaluation of an allogeneic CAM-based monocusp valve in ovine models following 7-day implantation.** (**A**) Representative images taken before, during, and after surgical implantation of CAM material in three sheep hearts to reconstruct the RVOT by the insertion of a monocusp valve and transannular patch. RV, right ventricle; PA, pulmonary artery; LV, left ventricle. *n*= 3. (**B**) Representative pressure recordings collected before (day 0) and after (day 0 and day 7) monocusp implantation. RVP, right ventricular pressure, yellow; PAP, pulmonary artery pressure, blue. *n*= 3. (**C**) Valvular pressure gradients before and immediately after monocusp implantation, and after seven days of implantation. *n* = 3. (**D**) The percentage of regurgitation, the cardiac output (CO), and the ejection fraction (EF) were estimated from ovine hemodynamic and magnetic resonance imaging (MRI) data collected on day 7. *n*= 3 (**E**) Representative echocardiography of the monocusp PVs in the ovine model viewed using in both long-axis (top) and short-axis (bottom) views. Double-sided white arrows indicate the characteristic longer length of the monocusp compared to the native leaflet. *n*= 3.

Fig. 6. **In vivo and biorobotic heart pressure waveforms are comparable. A**) Representative pressure waveforms for a monocusp valve over a cardiac cycle, comparing the biorobotic hybrid heart (labeled as ex vivo) and 7-day implantation in vivo ovine studies. RVP, right ventricular pressure; PAP, pulmonary arterial pressure. (**B**) Peak-to-peak differential pressure (dP = RVP - PAP) across the PV of native and monocusp valve scenarios ex vivo (n=2 hearts, three rounds of

testing, 30 consecutive cycles each) and in vivo physiological conditions (n=3 sheep). (**C**) Representative waveforms of the flow across the right ventricular outflow tract after in silico implantation of a transannular patch only or after monocusp valve implantation in silico and in vivo (n = 3 sheep). (**D**) Regurgitant volume across the transannular patch or monocusp conditions in different models. n = 3 sheep in vivo. Illustrations were created with Adobe Illustrator.

Fig. 7. CAM material integrates with native tissue with minimal signs of inflammation after 7 days of implantation. (**A**) Representative image of explanted and chemically fixed RVOT showing the monocusp, transannular patch, and native pulmonary leaflets in an intact condition. Explanted RVOT was opened longitudinally to visualize the monocusp and the two native pulmonary leaflets. PA: pulmonary artery and RV: right ventricle. The red dashed line indicates the approximative location of the longitudinal histological section of the monocusp and the transannular patch in (**B**). Hematoxylin-eosin-saffron staining of the cross-section of the implantation site (beige, collagen; pink, non-collagenous proteins and cytoplasm; and blue, cell nuclei). Low magnification cross-section of transannular patch, monocusp, and RV (left, red dashed outline; Scale bar, 5 mm). Histology of the edge and the root of the monocusp valve (center, cyan, and blue dashed outline; Scale bars, 500 μm). Histology of the middle of the monocusp and the middle of the transannular patch (right, purple and orange dashed outline; Scale bars, 500 μm. *n* = 3 sheep. (**C**) Representative immunostaining of the middle of the monocusp for α-SMA (red) and calponin (green), or CD3 (red) and CD45 (green) within the fibrin layer deposited at the surface of the CAM material (delimitated by the white dotted lines). Blue, nuclei. Scale bars, 50 μm. *n* = 3 sheep. (**D**) Immunostaining for $CCR7^+$ M1 macrophages

(white arrows) at the junction between the CAM material and the native tissue (delimited by the white dashed lines) with CD64 (red) and CCR7 (green) antigens. Co-localization of the Hoechst (nuclei in blue), calponin (green), and α-SMA (red) markers appears as white. Scale bar, 100 μm. n = 3 sheep.