Title: BIOMIMETIC POLYMERIC COMPOSITE FOR HEART VALVE REPAIR

Abstract: A biomimetic, polymeric composite biomaterial designed as a heart valve leaflet substitute that can be used for heart valve repair and/or to fabricate a new-generation of durable heart valve prosthesis.
BIOMIMETIC POLYMERIC COMPOSITE FOR HEART VALVE REPAIR


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FIELD

[3] A biomimetic, polymeric composite biomaterial designed as a heart valve leaflet substitute that can be used for heart valve repair and/or to fabricate a new-generation of durable heart valve prosthesis. In some embodiments, the polymeric composite biomaterial is in the form of a patch.

BACKGROUND

[4] Valve replacement in adults and children has inherent problems associated with anticoagulation (mechanical valves) or durability (bioprosthetic heart valves), which leads to the failure of the prosthesis and increases the probability for reoperation and the accompanying risk. Thus, valve repair is always the preferred approach, compared to replacement.

[5] Valve repairs frequently require the use of cardiovascular patches to perform leaflet augmentation or extension. Current available patches used for valve repair, such as bovine pericardium, porcine intestinal submucosa extracellular matrix, expanded
polytetrafluoroethylene, fresh autologous pericardium and glutaraldehyde-treated autologous pericardium, all have intrinsic limitations and drawbacks that affect their long-term durability and mechanical performance, leading to structural degeneration (SD) of the patch and of the repaired valve leaflet.


[7] To overcome these drawbacks, implantable patches or grafts with native-like structures and tunable mechanical performance close to the ones of the native valve leaflets were
attempted. In this regard, a polyvinyl alcohol (PVA)-bacterial cellulose (BC)-based hydrogel was designed to mimic the mechanical properties of the native valve leaflet. But, the degradable nature of PVA over time, poor design of the patch and lack of data on the durability of the composite hindered its application as a stable cardiac patch. Another composite fabrication involved the combination of poly(ethylene glycol) (PEG) hydrogel and polycaprolactone (PCL) fiber for heart valve tissue engineering, but this composite material demonstrated an anisotropic behavior on the unicycle tensile test only and had a linear stress-strain behavior that was different from the non-linear behavior of native leaflets. This may cause valvular interstitial cells (VICs) to experience greater stresses, impact VIC activation and extracellular matrix (ECM) remodeling, leading to calcification.

Masoumi et al. attempted a tri-layered scaffold designed to mimic structural and anisotropic mechanical characteristics of the native leaflet. (Masoumi, N. et al., A Tri-Layered Elastomeric Scaffolds for Engineering Heart Valve Leaflets. Biomaterials 2014, 35 (27), 7774–7785). But this biodegradable patch degraded at a fast rate with a loss of mechanical strength from 3.02 MPa to 1.63 MPa in 4 weeks. In comparison, native valve aortic and pulmonary leaflets keep a stable modulus of, 3.84 and 2.55 MPa, respectively over time. Despite aiming at replicating the architecture of native leaflets, Masoumi’s patch is not a mechanically stable option for clinical use. Thus, there remains a need for a stable, functional and biomimetic patch that overcomes the drawbacks of previous patches, including a clinical need for a new type of patch that can achieve a better durability after implantation in patients.
SUMMARY

[9] In one aspect, a stable biomimetic polymeric biomaterial is provided. The biomaterial includes at least two layers including a Fibrosa-mimic (“F-mimic”) layer, a Spongiosa-mimic (“S-mimic”) layer, and a Ventricularis-mimic (“V-mimic”) layer. In some embodiments, the F and V layers are anisotropic and the S layer is a shock absorbing layer. In some embodiments, the F-mimic layer and the V-mimic layer are made of polycarbonate polyurethane (PCU) film, enhanced with aligned, electrospun polycaprolactone (PCL) fibers, and the S-mimic layer is made of PCU foam. In some embodiments, the stable biomimetic polymeric biomaterial includes two to five layers. The stable biomimetic polymeric biomaterial may be devoid of animal-derived tissue, thus, in some embodiments it has no animal-derived tissue. The biomaterial may be used to make a patch, such as for treating a heart defect, or a prosthetic heart valve.

[10] In another aspect, a polymeric, biomimetic customized biomaterial patch (“BCP”) that replicates the structure-function driven architecture of native valve leaflets is provided and described herein. In one embodiment, the BCP replicates the three-layer architecture and the anisotropic mechanical properties of a native leaflet. Thus, in one embodiment, the BCP comprises a composite body including three polymeric layers. In this regard, the layers include a Fibrosa-mimic (“F-mimic”) layer; a Spongiosa-mimic (“S-mimic”) layer; and a Ventricularis-mimic (“V-mimic”) layer. In some embodiments, the F and V layers are anisotropic and the S layer is a shock absorbing layer. In some embodiments, the F-mimic layer and the V-mimic layer are made of polycarbonate polyurethane (PCU) film, enhanced with aligned, electrospun polycaprolactone (PCL) fiber mesh, and the S-mimic layer is made of PCU foam. In some
embodiments, the biomimetic patch is entirely polymeric, i.e., lacks animal-derived tissue. The tri-layered patch can be modified and tuned to achieve the specific mechanical requirement, as well as have a low antigenicity and lower risk for structural valve degeneration.

[11] The BCP described herein, as compared to three commercial patches, exhibits an anisotropic mechanical behavior and mechanical stiffness (6.20 ± 1.83 MPa and 1.80 ± 0.21 MPa in circumferential and radial directions, respectively), which is more similar to the native aortic valve leaflets than any currently available commercial patches. The BCPs also exhibit greater durability and greater biocompatibility. In vivo rat subcutaneous tests also confirmed the BCP exhibits mechanical biostability and superior resistance to inflammation and calcification, compared to the commercial patches. Thus, the BCP embodied herein provide a new clinical-grade biomaterial patch useful for heart valve extension, augmentation or replacement in children and adults.

[12] In yet another aspect, a novel polymeric valved device, such as an implantable prosthetic heart valve is provided. The implantable prosthetic heart valve comprises the biomaterial described herein. The implantable prosthetic heart valve may be an aortic valve, mitral valve, or tricuspid valve.

[13] In yet a further aspect, methods for repairing a heart defect with the BCP and methods for delivering an implantable heart valve to a subject in need thereof is described and embodied herein.

**BRIEF DESCRIPTION OF THE FIGURES**

[14] FIG. 1A shows a cross sectional view of the architecture of native heart valve;
[15] FIG. 1B shows a cross sectional view of a tri-layered biomimetic patch in accordance with one embodiment of the disclosed subject matter;

[16] FIG. 1C shows a cross sectional view of the V-mimic layer with aligned fibers in accordance with the embodiment in FIG. 1B in accordance with the disclosed subject matter;

[17] FIG. 1D shows a cross sectional view of an embodiment of the biomaterial having aligned PCL fibers in the F-mimic layer and V-mimic layer in accordance with the disclosed subject matter;

[18] FIG. 1E shows a top view and side perspective of the biomaterial including an S-mimic foam layer and a plurality of aligned fibers in accordance with the disclosed subject matter;

[19] FIG. 1F is a chart showing the tensile properties of the biomaterial of FIG. 1E compared to native leaflets in accordance with the disclosed subject matter;

[20] FIGS. 2A -2G show comparative mechanical properties of native leaflets tissue and commercial cardiac patch representatives in accordance with the disclosed subject matter;

[21] FIGS. 3A – 3I show structures and mechanical behaviors of electrospun fibers, fiber-enhanced layers, S-mimic layers and composite patches in accordance with the disclosed subject matter;

[22] FIG. 4 shows speckled specimen was glued and embedded between the plates in accordance with the disclosed subject matter;
[23] FIG. 4B shows schematic of pressure loading regimen in accordance with the disclosed subject matter;

[24] FIG. 4C shows definition of the specimen coordinate system for tissue samples in accordance with the disclosed subject matter;

[25] FIG. 4D shows results of cyclic loading for representative specimen in accordance with the disclosed subject matter;

[26] FIGS. 4E – 4G show contours of displacement components in X, Y, and Z directions at the maximum pressure in accordance with the disclosed subject matter;

[27] FIG. 4H show results of the representative cycle of HAV deformation in accordance with the disclosed subject matter;

[28] FIG. 5A shows an example of suture retention strength and thickness-normalized suture retention strength in accordance with the disclosed subject matter;

[29] FIG. 5B is a schematic representation of a specimen during suture retention strength test;

[30] FIG. 5C shows representative SRS curves of the commercial patches, the PCU films and the BCPs in accordance with the disclosed subject matter;

[31] FIG. 5D shows the SRS difference found among the commercial patches, the PCU films and the BCPs in accordance with the disclosed subject matter;

[32] FIG. 5E shows representative TN-SRS curves of the commercial patches, the PCU films and the BCPs in accordance with the disclosed subject matter;
FIG. 5 F shows the TN-SRS difference found among the commercial patches, the PCU films and the BCPs in accordance with the disclosed subject matter;

FIG. 6 A – 6 C shows comparative biostability performance of three commercial patches, the PCU films/foams and BCPs in accordance with the disclosed subject matter;

FIG. 7A and 7B shows biocompatibility performance: BSA protein adsorption and Ca\(^{2+}\) adhesion of commercial patches, the PCU film and the BCP in accordance with the disclosed subject matter;

FIG. 8 A-8D show histological characterization (H&E, Alizarin Red), mechanical property and calcium quantification of the PCU film, Gore-Tex® patch and CardioCel® Patch after in vivo implantation in accordance with the disclosed subject matter; and

FIG. 9 is a graph showing commercial patches have a general stiffer performance than native tissues and BCPs. The flexural modulus, calculated from the bulge tests, displays a trend in accordance with the tensile modulus from the tensile tests, especially the one obtained in C/H direction (orange line vs red bar) in accordance with the disclosed subject matter.

**DETAILED DESCRIPTION OF EXEMPLARY EMBODIMENTS**

In one aspect, a biomimetic polymeric biomaterial is provided. The biomimetic polymeric biomaterial is useful as a heart valve leaflet substitute and/or to fabricate a prosthesis. In one embodiment, the polymeric biomaterial is used to make a biomimetic customized biomaterial patch or BCP. In other embodiments, the biomimetic polymeric biomaterial is used to fabricate a polymeric valve prosthetic device. Thus, the biomimetic polymeric biomaterial may be used for treating a subject in need of heart valve repair and/or heart valve replacement.
[39] Generally, the BCP comprises a body having a multi-layered polymeric composite biomaterial. For example but not limitation, the multi-layered polymeric composite biomaterial may include two to five layers. In one embodiment, the biomaterial is a tri-layered polymer composite. In this embodiment, the BCP is designed to mimic the architecture, i.e., three distinct tissue layers that compose the valve leaflets, and the mechanical properties of native leaflet tissue.

[40] Referring to FIG. 1A, the architecture of the native heart valve 1000 is shown. The native heart valve tissue has a highly specialized architecture with three specific layers: the Fibrosa 1001, Spongiosa 1002, and Ventricularis 1003. They are composed of collagen, elastin and glycosaminoglycans (GAGs). The Fibrosa 1001 consists mainly of a dense network of corrugated type-I collagen fibers arranged in the circumferential direction, which provides the primary load-bearing properties of the heart valve. The Spongiosa 1002 is composed of highly hydrated GAGs and proteoglycans (PGs) as well as loosely arranged collagen and elastin. It acts as a cushion, enabling shear between the two other layers during loading and unloading, and absorbing the load resulting in minimal stress on the leaflet itself. The Ventricularis 1003 is comprised of less organized collagen fibers and radially oriented elastin sheets. It helps reduce large radial strains during the high blood flow over the valves when they are fully opened. The complex, highly organized structure of the valves leads to specialized mechanical properties necessary to withstand high trans-valvular pressures and low flexural stiffness.

[41] Referring to FIG. 1B, the BCP 100 is illustrated. The BCP comprises a Fibrosa-mimic ("F-mimic") layer 101, the Spongiosa-mimic ("S-mimic") layer 102 and the Ventricularis-mimic ("V-mimic") layer 103. The F-mimic layer 101 and V-mimic layer 102 are fiber-enhanced layers, comprising aligned PCL fibers and PCU film. The PCL fibers are embedded in the PCU matrix and dried as a fibrous film composite. The S-mimic layer 103 is a PCU foam layer that replicates the load-bearing mechanical role played by the native spongiosa. The F and V layers
(101, 102) are anisotropic and mimic the mechanical properties of the native fibrosa 1001 and ventricularis 1002. The S layer 102 (foam) acts as a shock absorbing layer and has the same mechanical properties as the native spongiosa 1003. Thus, the BCP 100 replicates the heart valve leaflets’ complex structure 1000. Referring to FIG. 1C, the V-mimic layer includes aligned fibers having a different direction as the fibers in the F-mimic layer, thus, the alignment of the fibers in these layers mimic that of native tissue. In another embodiment, referring to FIG. 1D, the F mimic layer and V-mimic layers may have PCL fibers aligned in substantially the same direction.

42 Referring to FIG. 1E, in another embodiment, the biomaterial is a composite structure including the S-mimic foam layer and a plurality of polypropylene fibers embedded in the foam structure to form a composite biomaterial. The polypropylene fibers, each have a longitudinal body and when embedded in the foam S-mimic layer are spaced apart from each other at a distance of between about 1 and about 3 mm. As one of ordinary skill in the art may appreciate, the length of the polypropylene fiber depends on the application and in particular the size of the biomaterial or patch desired. For example, patches that have a length of about 9 cm will include polypropylene fibers having a length of about 9 cm or less. The plurality of polypropylene fibers include 2 to 4 fibers. The fibers can also have different sizes. For example but not limitation, the diameter range for the fibers may be between about 0.030 to about 0.100 mm. In one embodiment, for example, the polypropylene fibers are monofilament sutures having sizes of 6-0, 7-0 and/or 8-0 (USP designation).

43 It has been found that the biomaterial comprising the S-mimic foam layer and a plurality of polypropylene fibers embedded in the foam structure to form a composite biomaterial offers mechanical properties substantially the same as native leaflet tissue, as shown in Table 1A below. As shown, the biomaterial in some embodiments exhibits a tensile modulus in a C/H direction of about 8 to about 16 MPa.
Table 1A.

Tensile Properties of Modified Version of BCPs and Native Leaflets

<table>
<thead>
<tr>
<th>Sample</th>
<th>Tensile Modulus (MPa) C/H Direction</th>
<th>Strain (%) where to obtain the tensile modulus</th>
<th>Tensile Modulus (MPa) R/V Direction</th>
<th>Strain (%) where to obtain the tensile modulus</th>
</tr>
</thead>
<tbody>
<tr>
<td>Human Aortic Valve Leaflet (HAV)</td>
<td>16.34 ± 0.42</td>
<td>14%-15%</td>
<td>0.03 ± 0.01</td>
<td>39%-40%</td>
</tr>
<tr>
<td>Suture 6-0 foam composite</td>
<td>15.27±0.05</td>
<td>14%-15%</td>
<td>0.58±0.005</td>
<td>39%-40%</td>
</tr>
<tr>
<td>Suture 7-0 foam composite</td>
<td>7.84±0.15</td>
<td>14%-15%</td>
<td>0.67±0.002</td>
<td>39%-40%</td>
</tr>
</tbody>
</table>

[44] Referring to FIG. 1F, various embodiments of the biomaterial are compared in a polypropylene suture – PCU foam composite to native leaflet tissue. As shown, the biomaterial may include polypropylene suture 7-0-foam 15% - Horizontal (H), polypropylene suture 7-0-foam 40% - Vertical (V), polypropylene suture 6-0-foam 15% - H and polypropylene suture 6-0-foam 40% - V. For example, a first embodiment is a 6-0 suture-PCU foam composite and a second embodiment is a 7-0 suture-PCU foam composite. Three samples shown in Table 1A, (2 suture-foams and 1 native tissue) were tested from two directions, i.e., the H/C direction and V/R direction. As used herein, H is the horizontal direction of a 2D biomimetic patch; it is used to represent similar direction, circumferential direction of 3D native leaflets. So, H=C. Similarly, V=R. Referring back to FIG. 1F, a comparison of the solid curves representing the suture 7-0 foam composite, suture 6-0 foam composite and HAV in the H/C direction, they are relatively close. Additionally, the dash curves representing suture 7-0 foam composite, suture 6-0 foam composite and HAV in the H/C direction in the V/R direction, are also close. These data
indicate that the embodiments of the suture foam composite are much more similar to native tissue than other commercial patches (Fig 2G).

[45] Exemplary Materials and Method for fabricating embodiment of FIG. 1 D.

[46] Carbothane™ AC-4075A, Polycarbonated-based polyurethane (PCU) was ordered from Lubrizol. Dimethylacetamide (DMAC) was purchased from Acros organics and used as the solvent to dissolve PCU. Polycaprolactone (PCL, Mw=80,000) was purchased from Sigma-Aldrich. Chloroform and methanol with 3:1 molar ratio, was used to dissolve PCL and prepared as the electrospinning solution. Commercially available patches including Gore-Tex® (W. L. Gore and Associates, Flagstaff, Arizona, USA), CorMatrix® (Cardiovascular, Inc, Atlanta, Georgia, USA) and CardioCel® (Admedus, Toowong, Queensland, Australia), porcine heart valves (obtained from a local slaughterhouse), and the CryoValve® aortic human valve (CryoLife Inc., Kennesaw, Georgia, USA) were used as controls. Leaflets from porcine valves and human homograft were dissected and kept intact in PBS.

[47] The BCP 100 was prepared by a combination of three native-tissue mimicking layers, respectively named the Fibrosa-mimic 101 (F-mimic) layer, the Spongiosa-mimic (S-mimic) layer 102 and the Ventricularis-mimic (V-mimic) layer 103. The F-mimic layer and V-mimic layer were designed as fiber-enhanced layers, composed of aligned PCL fibers and PCU film in order to replicate the anisotropy of these layers. The S-mimic layer was designed as a PCU foam to replicate the load-bearing mechanical role played by the native spongiosa. The structure of this BCP is shown in FIG. 1D. As shown, FIG. 1D has aligned PCL fibers in both the F-mimic layer and the V-mimic layer. As shown, fibers are in the same direction in the F and V-mimic layers.

[48] Fabrication of the F-mimic 101 and the V-mimic 102 fiber enhanced layers.
[49] Using a 15% PCL solution prepared in a mixed solvent (Chloroform: Methanol=3:1), PCL fibers were produced by electrospinning with the following parameters: a flow of 1 ml/hour, a voltage of 20 kV voltage and a distance of 15 cm between the nozzle and drum collector. The solution was spun towards a rotating collector at a rate of 1600 rpm to collect the aligned fibers. The fibers were allowed to dry overnight in a chemical hood for solvent evaporation before the following fabrication and characterization. The collected, aligned PCL fibers were embedded in solution-casted PCU film. The 15% PCU solution was casted by a doctor-blade coater through a 500 μm gap to control the film thickness. The fiber-solution composite was cured overnight in a chemical hood to evaporate the solvent and form the fiber-enhanced layers.

[50] Fabrication of the S-mimic layer 103.

[51] To produce the S-mimic layer, the 15% PCU solution was casted by a doctor-blade coater to create a film with a fixed thickness of 1500 μm. Subsequently, the film was immersed in deionized water for 24 hours. Then, the solvent-exchanged PCU film was frozen under -80°C. Lyophilization was conducted on the frozen PCU film at 0.1 mBar, -40°C for 72 hours and turned into a porous layer to work as the S-mimic layer.

[52] Fabrication of the BCP 100.

[53] The F-mimic layer was casted to form the fiber-enhanced layer. After 1 hour drying in the hood, the S-mimic layer was put over the casted composite and dried with the fiber-enhanced layer together in the chemical hood. Then this two-layer composite was put over the V-mimic layer to fabricate the BCPs.

[54] Morphology Characterization
[55] To characterize the PCL aligned fibers, each mimic layer and the BCPs, the specimens were sputter coated with gold/platinum and imaged with a Zeiss Sigma VP scanning electron microscope (SEM) at an accelerating voltage of 3 kV. SEM images were used for the visual inspection of fiber’s orientation, mimic layer and BCPs’ inner structures and their surface quality.

[56] Tensile mechanical testing

[57] The mechanical properties were measured with an Instron 5848 mechanical tester with a 50 N load cell at a strain rate of 10% s⁻¹. The specimens were cut as 5 mm x 20 mm stripes (for non-tissue samples) or 3 mm x 10 mm ones (for the native tissue samples) in two different directions, horizontally/circumferentially (H or C direction) and vertically/radially (V or R direction), shown in Fig 2B. The thickness of the specimens was measured at three different points with a digital caliper (Mitutoyo America Corp, Aurora, IL, USA) and the values were averaged. Four to six specimens for each samples were repeatedly stretched for 20 cycles, either to a maximal strain of 15% in H/C direction or to a maximal strain of 40% in V/R direction. Missirlis and Chong, Brewer et al. and Thubrikar et al. have all reported in vivo AV leaflet strains to be approximately 10-15% and 30-40% in the circumferential and radial directions, from systole to diastole respectively. After the first 5 preconditioning cycles, the subsequent 15 cycles of stress-strain curves were recorded and averaged and the tensile modulus $E$ were calculated as the Equation 1 below:

$$ E = \frac{\Delta \sigma}{\Delta \varepsilon} \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots [1] $$

[58] where $\Delta \sigma = \frac{\Delta F}{w_0 T_0}$ and $\Delta \varepsilon = \frac{\Delta l}{l_0}$ are engineering stress and engineering strain. $l_0, w_0,$ and $T_0$ are the dimension (length, width and thickness) of the specimen, $\Delta l$ is the change of elongation in length, and $\Delta F$ is the change of the force. Then the average curves and the tensile modulus at
the strain of 15% or 40% were used to compare the mechanical performance in different directions and to assess anisotropy. Then the average curves and the tensile modulus at the strain of 15% or 40% were used to compare the mechanical performance in different directions and to assess anisotropy.

[59]  Flexural mechanical testing

[60]  Flexural properties of the commercial patches, leaflet tissues and BCPs were tested via the bulge tests. All samples were pre-cut as the circular planar specimens using fine dissectors. Thickness was evaluated by averaging three measurements taken at specimen’s center with a digital caliper (Mitutoyo 547-526s). The diameter of the caliper’s contact plate was 10 mm, which was larger than the circular test area with a diameter of 6 mm; thus the specimens were assumed to be uniform in thickness. The specimens were speckled with black India ink to allow for DIC deformation tracking. The specimens were then glued between two plates with holes of 6 mm diameter (Fig.4A). The embedded specimen was secured onto a custom inflation chamber through the holder.

[61]  The specimens were inflated by a custom-made displacement-driven syringe injection of PBS into the custom-made pressurization chamber. The pressure was monitored by a pressure transducer with 0-8 kPa range. The loading regimen was programmed using LabView (V2020, National Instruments, Austin, TX) and displayed in Fig 4B. The specimen was brought to a baseline pressure of 0.2 kPa and held for 30 seconds prior to cyclic testing to ensure the specimen was at equilibrium. The specimens were subjected to 30 load-unload cycles at a rate of 3.5 kPa/s from the baseline pressure to a maximum pressure of 7.2 kPa. These cycles were used to mimic the deformation under the quasi-physiological pressure level.
[62] The deforming specimen surface was imaged by two stereoscopically arranged cameras with 20 mm focus lengths at an aperture of f/4. The optical axes of the cameras were positioned 35 cm above the chamber and fixed with a total angle of 12\(^\circ\). This configuration had a depth of field in front over 1.5 cm, sufficient to capture the deformation of the specimen between 0.2–7.2 kPa. Images were collected during testing at a rate of 10 Hz by VicSnap 2009 and correlated by Vic3D (V8, Correlated Solution, Inc. Columbia, SC, USA).

[63] To calculate the pressure and displacement resultants, the measured pressure and displacement were tared by the baselines, resulting in relatively zero stress and strain at the reference state. The method of calculating the flexural modulus \(E_{flex}\) is provided below. The sample in this test was modeled as a circular thin plate with edges fully fixed. The pressure was evenly distributed on the bottom surface of the sample. The governing equation and boundary conditions of this case could be expressed in cylindrical coordinates \((r, \theta, z)\) as

\[
\nabla^2 \nabla^2 w = - \frac{\Delta P}{D}
\]

\[\text{s.t. } w(R) = 0, \quad \phi(R) = 0\]  \[\text{[2]}\]

where \(w\) is the displacement of \(z\) direction (defined as the out-of-plane direction) at a point of the thin plate, \(R\) is the radius of the plate, and \(\Delta P\) is the pressure exerted. \(D\) is the flexural rigidity defined as \(\frac{E_{flex}T_0^3}{12(1-\nu^2)}\). \(T_0\) is the thickness of the specimen. The solution to this equation is

\[
w(r) = - \frac{\Delta P}{64D} (R^2 - r^2)
\]

\[\text{[3]}\]

At the center point \((r = 0)\), the flexural modulus could be expressed as
\[ E_{\text{flex}} = \Delta P \frac{3R^4(1 - v^2)}{16T_0^3\Delta W} \]...

where \(\Delta W\) is the change of the displacement in z-direction. Here, all the materials were assumed to be incompressible, so the Poisson’s ratios were all set as 0.5.

[64] Suture retention testing

[65] The suture retention capabilities of the three commercial patches, PCU films and the BCPs were tested following the steps described in Pensalfini et al.’s work\(^2\), using Instron 5848 tensile machine (Fig 5A). Prolene 5-0 suture was inserted 2 mm from the end of the 10 x 15 mm specimen and through the specimen to form a half loop. The suture was pulled at the rate of 50 mm/min crosshead speed (Fig 5B). Five specimens were tested in each group. The force (N) required to pull the suture through and/or cause the specimen to fail was recorded as the suture retention strength (SRS). A thickness normalized suture retention strength (TN-SRS, N/mm\(^2\)) was calculated, by dividing the suture retention strength by the area of the sample over which the load was applied\(^3\):

\[ \text{TN - SRS} = \frac{\text{SRS}}{\text{Suture Thread Diameter} + \text{Sample Thickness}} \]...

and compared among all the samples.

[66] Biostability testing

[67] Specimens of the commercial patches, PCU films/foams and BCPs were pre-cut as 5 mm x 30 mm and submerged into 2 mL vials filled with an \textit{in vitro} solution of 20% hydrogen peroxide (H\(_2\)O\(_2\))/0.1M cobalt chloride (CoCl\(_2\)). The \textit{in vitro} solution was refreshed twice a week, and all testing were done at 37°C. After a period of 5, 10, 14, 15, 20, 24, and 30 days, the specimens were removed, rinsed thoroughly in deionized-water, dried in the hood, then cut into two parts (5 mm x 25 mm and 5 mm x 5 mm). The former was tested via the tensile mechanical
testing and the tensile modulus at strain=15% was calculated. The latter was analyzed by SEM to inspect the surface quality.

[68] Biocompatibility testing

[69] Bovine Serum Albumin (BSA) static protein-adsorption experiments.

[70] For static protein-adsorption tests, 1 mg mL\(^{-1}\) BSA solution was prepared in PBS (pH 7.4). Commercial patches and PCU films were cut into specimens (50 mm x 10 mm) and immersed in 10 mL 1 mg mL\(^{-1}\) BSA solution in a test tube. BSA adsorption was conducted under vibration at 37°C for 3 hours to allow for adsorption equilibrium. Then the specimens were rinsed with PBS, the remaining proteins adsorbed on the surfaces were removed with a 1 wt% aqueous solution of sodium dodecylsulfate (SDS), similar to the work done by Song et al.\(^{24}\). The experiments were performed with five measurements for each specimen. BSA content was measured using a NanoDrop\textsuperscript{TM} spectrophotometer at a wavelength of 280 nm and then the amount of adsorbed BSA on specimens was calculated.

[71] Calcium-ion (Ca\(^{2+}\)) adhesion experiments.

[72] The Ca\(^{2+}\) adhesion experiments were performed in a metastable calcium phosphate (MCP) solution. The purpose of using this MCP solution is to obtain calcium-phosphate compounds which can precipitate out from the solution and deposit on the tested specimens, in order to test the samples’ calcification resistance in \textit{in vitro} studies\(^{25}\). Similar experiments were performed as reported earlier\(^{26}\). In brief, 3.87 millimole (mM) CaCl\(_2\), 2.32 mM K\(_2\)HPO\(_4\), and 0.05M Tris buffer were solved in 1000 ml of de-ionized water, to yield a Ca/PO\(_4\) ratio of 1.67.

[73] This solution is more physiologically representative of hydroxyapatite, with a Ca/PO\(_4\) ratio of 1.67, which is the most common form of calcium minerals in the vascular calcification process. Commercial patches and PCU films were cut into specimens (5 mm x 30 mm) and
immersed in 2 mL MCP solution individually. This experiment was conducted under vibration at 37°C and solution was changed every 48 hours to ensure an adequate ion concentration. The specimens were removed after 16 days and rinsed with water to remove excess solution and loosely attached deposits. The specimens were dried in the vacuum oven at 70°C overnight, accurately weighed, and hydrolyzed in 2 mL of 2 N HCl for 24 hours at 50°C. The calcium concentration was determined from HCl hydrolysate, using calcium colorimetric assay.

[74] Rat subcutaneous implant model. In accordance with NIH guidelines for the care and use of laboratory animals (NIH Publication #85-23 Rev. 1985), all animal protocols were approved by the Institutional Animal Care and Use Committee (IACUC) of Columbia University (Protocol #AC-AABD5614).

[75] Eighteen specimens (diameter =8mm) of PCU film (n=6), Gore-Tex® patch (n=6) and CardioCel® patch (n=6) were implanted in the subcutaneous position of three rats. Following induction of anesthesia, fur clipping, and standard sterile prepping and draping, six subcutaneous pockets were created on the dorsal surface of each rat. One specimen was implanted into each pocket, after which all wounds were re-approximated with surgical clips. The rats were sacrificed at 8 weeks with an overdose of isoflurane (Euthenase).

[76] Histology.

[77] The implanted specimen was retrieved while still contained in host tissue, fixed in 10% neutral buffered formalin and processed using paraffin-embedding techniques. Slides were stained with Hematoxylin and Eosin and Alizarin Red stains. In each specimen, both the patch and the surrounding host tissue were evaluated.

[78] Calcium Content & mechanical test.
[79] Samples were analyzed for calcium content using calcium colorimetric assay as described in Calcium-ion adhesion experiments described above. Briefly, the specimen disks were removed from host tissue, fixed in formalin and solvent-exchanged in DI-water. Following with the lyophilization, the net weight of the specimen disks were acquired. After hydrolyzing in nitric acid, the calcium content was quantitated. Results are reported as microgram calcium per milligram dry specimen weight. The PCU disks can be separated from the host tissue after lyophilization. This specimen’s mechanical performance was also evaluated as described in section 2.4 and its tensile modulus at the strain=15% was recorded, to compare with the control, unplanted sample.

[80] Statistical Analysis. Statistical analyses of the tensile mechanical properties, biostability mechanical tests, protein adsorption and calcium adhesion tests were performed using one-way analysis of variance (ANOVA). P values less than 0.05 were considered statistically significant (*P<0.05, **P<0.01, ***P<0.001 and ****P<0.0001). And differences between samples within the groups were evaluated using a student’s t-test, or Tukey’s multiple comparisons test followed by ANOVA. GraphPad Prism 7 (San Diego, CA, USA), statistics package, was used to obtain statistical significance for the study above.

[81] Results

[82] Structure and Mechanical Properties

[83] Tensile properties of native tissues and commercial patches. We performed cyclic, uniaxial tensile tests on native leaflets and commercial patches in order to compare the mechanical performance of our BCP with these reference tissues (Fig.2). For native leaflets samples, after the first 5 pre-conditioning tensile cycles, the average of the up-curves from the subsequent 15 cycles exhibit a residue elongation and then an increase in the slope of the stress-strain curves which is attributed to the deformation and stretch of fiber networks in the tissue.
This increase is accentuated in the circumferential direction (C-direction) compared to the radial direction (R-direction) due to the existence of oriented, crimped collagen fibers in circumferential direction (Fig. 2C). The tensile modulus was calculated using the equation 1 at the strain of 15% and 40%, for the C-direction and R-direction respectively. It shows that the human aortic valve leaflets (HAVs) have a higher tensile modulus value, 16.34±0.42 MPa, than the value of porcine aortic valve leaflets (PAVs), 8.71±9.88 MPa, at the strain=15% in C-direction. For same species, porcine pulmonary valve leaflets (PPVs) are much stiffer than PAVs, 20.00±13.41MPa vs 8.71±9.88 MPa in C-direction and 0.52±0.85 MPa vs 0.19±0.20 MPa in R-direction (Table. 1 and Fig.2C). All these human and porcine leaflets display a highly anisotropic performance and are much stiffer in the C-direction than the R-direction.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Tensile Modulus (MPa)</th>
<th>Strain (%) where to obtain the tensile modulus</th>
<th>Tensile Modulus (MPa)</th>
<th>Strain (%) where to obtain the tensile modulus</th>
</tr>
</thead>
<tbody>
<tr>
<td>Human Aortic Valve Leaflet</td>
<td>16.34 ± 0.42</td>
<td>14%-15%</td>
<td>0.03 ± 0.01</td>
<td>39%-40%</td>
</tr>
<tr>
<td>Porcine Aortic Valve Leaflet</td>
<td>8.71 ± 9.88</td>
<td>14%-15%</td>
<td>0.19 ± 0.20</td>
<td>39%-40%</td>
</tr>
<tr>
<td>Porcine Pulmonary Valve Leaflet</td>
<td>20.00 ± 13.41</td>
<td>14%-15%</td>
<td>0.52 ± 0.85</td>
<td>39%-40%</td>
</tr>
<tr>
<td>Gore-Tex® Patch-(H)</td>
<td>85.04 ± 41.27</td>
<td>14%-15%</td>
<td>181.85 ± 55.50</td>
<td>39%-40%</td>
</tr>
<tr>
<td></td>
<td>Tensile modulus</td>
<td>Strain</td>
<td>Ultimate tensile strength</td>
<td>Elongation in fracture</td>
</tr>
<tr>
<td>------------------</td>
<td>-----------------</td>
<td>--------</td>
<td>---------------------------</td>
<td>------------------------</td>
</tr>
<tr>
<td>Gore-Tex® Patch-(V)</td>
<td>81.46 ± 22.28</td>
<td>14%-15%</td>
<td>179.89 ± 22.00</td>
<td>39%-40%</td>
</tr>
<tr>
<td>CorMatrix® Patch-(H)</td>
<td>55.55 ± 26.72</td>
<td>14%-15%</td>
<td>35.53 ± 7.08</td>
<td>39%-40%</td>
</tr>
<tr>
<td>CorMatrix® Patch-(V)</td>
<td>39.52 ± 6.78</td>
<td>14%-15%</td>
<td>23.08 ± 12.81</td>
<td>39%-40%</td>
</tr>
<tr>
<td>CardioCel® Patch-(H)</td>
<td>13.17 ± 6.59</td>
<td>14%-15%</td>
<td>84.68 ± 29.05</td>
<td>39%-40%</td>
</tr>
<tr>
<td>CardioCel® Patch-(V)</td>
<td>10.88 ± 3.76</td>
<td>14%-15%</td>
<td>160.53 ± 25.41</td>
<td>39%-40%</td>
</tr>
</tbody>
</table>

[84] Mechanical characteristics of commercial patches (Gore-Tex®, CorMatrix® and CardioCel®) were obtained under the same conditions as the native tissues and are presented in Table 1. Compared to native tissues, the commercial patches are generally much stiffer, with a tensile modulus in the range of 6-120 MPa at strain=15% in C-direction and 23-180 MPa at strain=40% in R-direction. Commercial patches also display a non-anisotropic behavior, with similar tensile modulus at the same strain level in the horizontal (H) and vertical (V) directions (Table 1, Fig. 2D-G). And Gore-Tex® is the most isotropic and stiffest samples among these three commercial patches. CorMatrix® and CardioCel® are more compliant than Gore-Tex® and CardioCel® even has a similar tensile modulus as those of HAV at strain=15% in H-direction. They are also relatively anisotropic due to the nature of bio-based patches, with residue fibers in the product. Overall, the commercial patches still possess much stiffer tensile properties compared to HAV or any native leaflets, especially at the R/V direction. Without specific design, they are either randomly anisotropic (for CorMatrix® and CardioCel®) or isotropic (for Gore-Tex®).

[85] Referring to FIG. 2, which shows mechanical properties of native leaflets tissue and the commercial cardiac patch representatives. Tissue specimens were cut from homograft aortic valve and porcine aortic/pulmonary valves (FIG. 2A). The tissue leaflets were cut in circumferential direction and radial (R) direction to prepare specimens for the mechanical test (FIG. 2B). Stress-strain up-curves of HAV, PAV and PPV in C and R directions. Those are
average up-curves of 15 cycles after 5 cycles preconditioning. All tissue samples have a much stiffer tensile performance in C direction than R direction (FIG. 2 C). None of the three types of commercial patches (Gore-Tex®, CorMatrix®, and CardioCel®) displayed anisotropic mechanical properties (similar average curves in H and V directions) or similar range of tensile modulus close to HAV (FIGS. 2D-2F). And a detailed elastic modulus comparison illustrated the significant difference on mechanical stiffness, between commercial patches and HAV. (FIG. 2G) (*P<0.05, ***P<0.001 and ns=not significant).

[86] Structure and tensile properties of the mimic layers and the BCPs. FIG. 3 and Table 2 displayed the microscopic structure and mechanical properties of the PCL electrospun fibers, the mimic layers and the BCPs. As demonstrated by the SEM image (FIG. 3A), the aligned PCL fibers are electrospun with a highly-orientated distribution and exhibit a highly anisotropic performance during the cyclic tensile tests (35.74 ± 9.81 MPa vs 1.63 ± 0.38 MPa), compared to the random PCL fibers electrospun from the same solution (7.37 ± 0.30 MPa). The fiber-enhanced F-mimic and V-mimic layers also demonstrate an anisotropic behavior in two directions, due to the incorporation of the aligned PCL fibers (FIG. 3B, 3C). In the H direction, V-mimic and F-mimic layers show a combination of the properties of the PCU film (up-curve) and the PCL aligned fibers (FIG. 3B). While in the vertical direction, they had a similar behavior as the PCU films (FIG. 3E). The PCU properties were dominant and the PCL fibers played a less significant contribution in V direction. The fiber-enhanced layers tensile modulus are 33.38 ± 7.1 MPa at the strain=15% in the H direction and 2.55 ± 1.02 MPa at the strain=40% in the V direction: these layers exhibit an anisotropic behavior and are more compliant than most of commercial patches (except for CardioCel® in H direction) but are still stiffer than the native tissue.

[87] In order to increase the compliant of the overall composite, also to correspond to the Spongiosa layer, PCU foam was made via lyophilization. Compared to the film made from the
same concentration PCU solution, the foam exhibited a porous structure (Fig.3D), a more compliant mechanical behavior and a significant lower tensile modulus (0.55-0.59 MPa), shown in Fig.3E and 3F.

[88] The biomimetic, customized three-layered composite patch (BCP) was obtained by coating the fiber-enhanced layers on both sides of the S-mimic layer (Fig.3G). This BCP demonstrates an anisotropic mechanical behavior which is close to those of native human valve leaflets (Fig.3H). The BCP also has a tensile modulus of 6.20 ± 1.83 MPa at the strain=15% in the H direction and 1.80 ± 0.21 MPa at the strain=40% in the V direction. Compared to the CardioCel® patch with the best mechanical performance so far, the BCP has a lower stiffness in H direction and more compliant performance in V direction. From the mechanical viewpoint, it is more comparable to the native leaflets than the commercial patches, especially in V/R direction (Fig.3I, Table 1 and Table 2)

<table>
<thead>
<tr>
<th>Sample</th>
<th>Tensile Modulus (MPa) $C/H$ Direction</th>
<th>Strain (%) where to obtain the tensile modulus</th>
<th>Tensile Modulus (MPa) $R/V$ Direction</th>
<th>Strain (%) where to obtain the tensile modulus</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aligned PCL fiber</td>
<td>35.74 ± 9.81</td>
<td>14-15%</td>
<td>1.63 ± 0.38</td>
<td>39%-40%</td>
</tr>
<tr>
<td>Random PCL fiber</td>
<td>7.37 ± 0.30</td>
<td>39%-40%</td>
<td>7.37 ± 0.30</td>
<td>39%-40%</td>
</tr>
<tr>
<td>Fiber-enhanced Layer (F/V-mimic layer)</td>
<td>33.38 ± 7.1</td>
<td>14%-15%</td>
<td>2.55 ± 1.02</td>
<td>39%-40%</td>
</tr>
</tbody>
</table>
Referring to FIG. 3 showing structures and mechanical behaviors of electrospun fibers, fiber-enhanced layers, S-mimic layers and composite patches. The SEM of the aligned PCL fibers have illustrated fibers’ orientation (FIG. 3A). Compared to the random PCL fibers, the aligned PCL fibers demonstrated an anisotropic mechanical performance. The fiber-enhanced film (F/V-mimic layers) exhibited an anisotropic mechanical performance, stronger on the H direction (same as aligned fiber direction) and similar performance as pure PCU film on the V direction (perpendicular to the aligned fiber direction) (FIG. 3B-3C). The SEM image displayed the cross-section of the PCU foam: the porous structure and the layer structure (FIG 3D). The PCU foam has a significant lower elastic modulus (**P<0.0001) compared to the PCU film fabricated from the same solution (FIG. 3E-3F). The SEM image of the cross-section of the composite patch illustrated the tri-layer structure: Film-Foam-Film, which paralleled the design of our composite patch in Fig. 1B (FIG. 3 G). The BCP exhibited an anisotropic behavior and a relatively close mechanical performance to HAV in both C/H and R/V directions. Although it may not as stiff as CardioCel in C/H direction, it was more compliant in R/V direction to avoid severe mismatch issue (FIG. 3H-3I)

Flexural properties. Bulge tests were performed to assess the flexural properties of the HAV, commercial patches and the BCP disclosed and embodied herein. The bulge test measured the components of the displacements in a 3D coordinate (FIG. 4C), providing the U, V and W components of the displacement field in X, Y and Z directions. For tissue samples, the X axes were defined as the dominant fiber direction and the Y axes were the perpendicular direction. For commercial patches or BCPs, the X and Y axes were defined by the in-plain directions that
corresponded to the stiffest and most compliant performance, respectively. Preconditioning was found to have a negligible effect on the mechanic response. The fiber orientation and anisotropic degree was characterized and evaluated by the principal strains e1 and e2. And the flexural modulus was calculated through the change of the applied pressure (ΔP) and the change of the out-of-plane displacement component (ΔW).

[91] Preconditioning was found to have a negligible effect on the mechanical response. Fig.4D plots the strain variation at the direction of Z, for the whole 30 loading cycles for the human aortic valve sample. The averaged maximum strain in Z direction over the first 10 cycles was 288.10 ± 1.24%, and the averaged maximum strain over the whole 30 cycles was 289.64 ± 1.40%. It’s a 0.53% variation and indicates that preconditioning minimally affected the mechanical response of the tissue. Consequently, the 16\textsuperscript{th} curve was used as the average data to calculate the anisotropic degree and the flexural modulus.

[92] Anisotropic level. FIGS. 4 E-G plots the three displacements in X, Y and Z directions at the maximum pressure of the 16\textsuperscript{th} loading cycle and the change of e\textsubscript{1} and e\textsubscript{2} during the 16\textsuperscript{th} loading cycle for the human aortic valve sample as the demonstration representative (FIG 4H). The ratio between e\textsubscript{1} and e\textsubscript{2} was then defined as the anisotropic level. If the ratio is close to 1, the specimen behaves more like isotropic material. Otherwise, it behaves more like anisotropic material. FIG 4G exhibited that the contour of the out-of-plane displacement, W, formed concentric ellipses rather than concentric circles. It is also evidence to demonstrate its anisotropy since most of the specimens deformed from a circular sheet to an ellipsoidal dome indicating the presence of anisotropy. Deformation in U and V with different displacement range was also an indication to display the anisotropy of the human aortic valve tissue (FIG 4E & F)

[93] Referring to FIG. 4, a speckled specimen was glued and embedded between the plates (FIG. 4A). Schematic of pressure loading regimen. After 30s held under a pressure of 0.2 kPa,
the samples were loaded from the baseline pressure to a maximum pressure of 7.2 kPa at a rate of 3.5 kPa/s and return to the baseline pressure at the same rate. Total load-unload cycles were 30 (FIG. 4B). Definition of the specimen coordinate system. For tissue samples, X was defined as the dominant fiber direction, Y was defined as the perpendicular direction and Z was defined as the out-of-plane direction. For non-tissue samples, X and Y were defined as the directions with the stiffest and most compliant mechanical performance. Z was also the out-of-plane direction (FIG. 4C). Results of cyclic loading for representative specimen, HAV leaflets at a loading/unloading rate of 3.5 kPa/s. With the similar strain variation in Z direction, the preconditioning had a negligible effect on the long-term mechanic response (FIG. 4D). Contours of displacement components in X, Y, and Z directions at the maximum pressure. The anisotropy of the tissue was evident from the U and V contours and elliptical W contours (FIGS. 4E-4G). Results of the representative cycle of HAV deformation. The change of e1 and e2 were recorded to compare and assessed the anisotropy of the specimen (FIG. 4H).

Table 3 summarized the ratio of principal strain and second principal strain in-plane, e1/e2. Among the three commercial patches, the Gore-Tex® patch was the most isotropic one, and CorMatrix® is the most anisotropic. For native tissues, PPV and HAV have obvious anisotropic behaviors. For BCPs, although from the design and the tensile test data they were demonstrated as the anisotropic composites, the average ratio of e1/e2 is just higher than Gore-Tex®. It may be attributed to the similar scale of the tensile modulus in-plane X and Y directions.

Table 3 Flexural Properties of Native Tissues, Commercial Patches and BCPs

<table>
<thead>
<tr>
<th>Sample</th>
<th>Thickness (mm)</th>
<th>ΔW (mm)</th>
<th>ν</th>
<th>e1/e2</th>
<th>Eflex (MPa)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gore-Tex®</td>
<td>0.382 ± 0.011</td>
<td>0.120 ± 0.031</td>
<td>0.46</td>
<td>1.16 ± 0.13</td>
<td>17.58 ± 4.50</td>
</tr>
<tr>
<td>CorMatrix®</td>
<td>0.309 ± 0.109</td>
<td>0.366 ± 0.030</td>
<td>0.45</td>
<td>1.80 ± 0.68</td>
<td>10.52 ± 1.03</td>
</tr>
<tr>
<td></td>
<td>0.364 ± 0.101</td>
<td>0.722 ± 0.137</td>
<td>0.45</td>
<td>1.52 ± 0.13</td>
<td>4.52 ± 2.40</td>
</tr>
<tr>
<td>---------------</td>
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<td>-------------</td>
</tr>
<tr>
<td>PPV</td>
<td>0.271 ± 0.032</td>
<td>1.275 ± 0.266</td>
<td>0.45</td>
<td>2.10 ± 0.81</td>
<td>4.87 ± 1.59</td>
</tr>
<tr>
<td>PAV</td>
<td>0.480 ± 0.001</td>
<td>1.922 ± 0.077</td>
<td>0.45</td>
<td>1.29 ± 0.04</td>
<td>0.53 ± 0.02</td>
</tr>
<tr>
<td>HAV</td>
<td>0.347 ± 0.038</td>
<td>1.196 ± 0.472</td>
<td>0.45</td>
<td>1.70 ± 0.51</td>
<td>2.70 ± 1.30</td>
</tr>
<tr>
<td>BCP</td>
<td>0.688 ± 0.186</td>
<td>0.231 ± 0.166</td>
<td>0.33</td>
<td>1.26 ± 0.02</td>
<td>3.55 ± 2.80</td>
</tr>
</tbody>
</table>

All data is acquired from the 16th loading-unloading cycles during the bulge tests

[95] Flexural Modulus: Table 3 also summarized the data of thickness and displacement of specimens in the out-of-plane direction. It can be seen that the commercial patches generally possessed higher flexural modulus. Gore-Tex® was the stiffest among those three types of patches (17.58 ± 4.50 MPa) and CardioCel® was the most compliant one (4.52 ± 2.40 MPa). Native tissues, including porcine leaflets and human leaflets, behaved more compliant than commercial patches during the bulge tests. For BCPs, they had a similar flexural modulus range (3.55 ± 2.80 MPa) as HAV (2.70 ± 1.30 MPa), and displayed better compliance than Gore-Tex® and CorMatrix®.

[96] Suture retention: The resistance to tearing of the BCPs, the raw material (PCU film) and the three commercial patches were determined by suture retention strength measurements. The mean suture retention strength (SRS) of Gore-Tex®, CardioCel® and CorMatrix® are 5.35 ± 1.25 N, 8.99 ± 1.77 N and 4.07 ± 1.38 N respectively (Table 4). The SRS of the BCP and the PCU film are in the range of the commercial patches (FIGS 5C-5D). Moreover, there is no significant difference on SRS of composite patches in H and V directions, reflecting a uniform resistance to tearing on the whole patch.
A thickness-normalized SRS (TN-SRS) has also been applied to eliminate the effect of sample thickness and needle size. According to the Equation 5, the TN-SRS of Gore-Tex®, CardioCel® and CorMatrix® were 94.76 ± 22.14 N/mm², 98.26 ± 19.35 N/mm², and 82.86 ± 28.10 N/mm² respectively (Table 4). There’s no significant difference on TN-SRSs among those three commercial patches. TN-SRSs of the BCPs in two directions were 89.91 ± 13.25 N/mm², and 79.1 ± 11.1 N/mm² respectively and were not significantly different from the three commercial patches (FIG. 5F). It was noted as well that the BCPs had a longer elongation, around 20mm (FIG. 5E) than the commercial patches, which indicated that the composite patch had a higher toughness than other samples by simply integrating the stress-strain curves.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Thickness (µm)</th>
<th>SRS (N)</th>
<th>TN-SRS (N/mm²)</th>
</tr>
</thead>
<tbody>
<tr>
<td>PCU Film</td>
<td>242.5 ± 1.5</td>
<td>6.32 ± 1.02</td>
<td>175.53 ± 28.33</td>
</tr>
<tr>
<td>Gore-Tex® Patch</td>
<td>383 ± 4.9</td>
<td>5.35 ± 1.25</td>
<td>94.76 ± 22.14</td>
</tr>
<tr>
<td>CardioCel® Patch</td>
<td>603.3 ± 27.3</td>
<td>8.99 ± 1.77</td>
<td>98.26 ± 19.35</td>
</tr>
<tr>
<td>CorMatrix® Patch</td>
<td>344 ± 11.4</td>
<td>4.07 ± 1.38</td>
<td>82.86 ± 28.10</td>
</tr>
<tr>
<td>BCP (H)</td>
<td>509 ± 21.5</td>
<td>6.58 ± 0.97</td>
<td>89.91 ± 13.25</td>
</tr>
<tr>
<td>BCP (V)</td>
<td>509 ± 21.5</td>
<td>6.25 ± 0.88</td>
<td>79.1 ± 11.1</td>
</tr>
</tbody>
</table>

Referring to FIG. 5 shows Suture Retention Strength and Thickness-normalized Suture Retention Strength. Example (FIG. 5A) and schematic representation (FIG. 5B) of a specimen
during suture retention strength test: The main geometrical parameters and the tensile rate are defined. Representative SRS curves of the commercial patches, the PCU films and the BCPs (FIG. 5C). Comparison of all groups displayed that the BCP had a SRS in the range of the average level of the commercial patches. Although One-way ANOVA test displayed the difference (*p<0.05) between BCP and commercial patches, the following Tukey’s test verified no significant difference between BCP and each commercial patch. The difference was found among the commercial patches (FIG. 5D). Representative TN-SRS curves of the commercial patches, the PCU films and the BCPs (FIG. 5E). The TN-SRS of the BCP had no significant difference compared to the commercial patches (FIG. 5F).

[99] Biostability.

[100] The biostability of the commercial patches, PCU-based raw film/foam and our BCPs were assessed by an accelerated oxidative degradation test, using a 0.1 M CoCl₂/20% H₂O₂ solution. FIG.6 showed the results of the biostability tests applied to all samples. Two of the commercial patches, CorMatrix® and CardioCel®, which are derived from biological materials, fully degraded and dissolved in the oxidization solution within Day 1, while the polymer-based samples (Gore-Tex®, PCU film/foam and BCPs) display an excellent stability during the 30-day period (FIGS. 6A-D), no significant difference on the mechanical properties in 30 days (one-way ANOVA). Nevertheless, the SEM images of PCU films’ surface display the formation of oxidization spots and dents after 20-30 days (FIG. 6C), suggesting a potential that the BCPs, with PCU film as the outside layer, might be impacted by a long-term oxidization starting from the surface.

[101] Referring again to FIG. 6, biostability performance of three commercial patches, the PCU films/foams and BCPs is shown. The tensile modulus of polymer-based product, including PCU film/foam, Gore-Tex® and BCP remained stable throughout the 30 days in the accelerated
oxidization solution, as opposed to the commercial CardioCel® and CorMatrix® patches, demonstrating an excellent biostability for a duration equivalent to 15 months of in vivo implantation (FIG. 6A) Specifically, the PCU films and foams as the main component and BCP itself, showed a stable mechanical performance (no significance change on mechanical properties via One-way ANOVA) in 30 days (FIG. 6B1-6B3) SEM images unveiled details on the surface morphology, suggesting the beginning of a slow degradation process starting at the outside surface layer (FIG. 6C).

[102] Biocompatibility.

[103] BSA Protein Adsorption. A BSA protein adsorption test was applied to assess the blood compatibility of the three commercial patches and BCPs. FIG. 7A illustrates the amounts of adsorbed protein on the BCPs and three commercial patch surface. The two polymer-based patches (BCP and Gore-Tex®) showed similarly low adsorbed BSA amounts and no significant difference between the adsorption levels of these two samples. On the other hand, the two patches derived from biological tissues (CorMatrix® and CardioCel®), exhibit a much higher dose of adsorbed albumin compared to the BCPs (p<0.0001, One-way ANOVA). The BCPs, thus, has a low level of protein adsorption that compares favorably to the three commercial patches.

[104] Ca²⁺ Adhesion. Table. 5 shows the results of 16-day Ca²⁺ adhesion tests performed on the PCU film, BCP and commercial patches. It has clearly stated that the PCU film and BCP have a lower Ca²⁺ deposition compared to Gore-Tex® and CardioCel® patches (FIG. 7B). A similar trend has been observed in the calcification amounts from the 8-week in vivo rat subcutaneous test (Table 5). Overall, both of the BCP and PCU surface layer have a lower level of calcification compared to commercial patches in vitro tests. It also provides a solid foundation for further evaluation in vivo tests.
[105] Referring to FIG. 7 showing biocompatibility performance: BSA protein adsorption and Ca\textsuperscript{2+} adhesion of commercial patches, the PCU film and the BCP. The polymer-based sample, BCP and Gore-Tex\textsuperscript{®} patch had a significant lower amount of BSA protein adsorption compared to two biological materials-derived patches (****\textit{p}<0.0001). No significant difference on the capacity of BSA absorption between BCP and Gore-Tex\textsuperscript{®} (FIG. 7 A) In a 16-day \textit{in vitro} Ca-ion adhesion test, the PCU film and BCP had a lower Ca contents in unit of dry samples, compared to the Gore-Tex\textsuperscript{®} patch and the CardioCel Patch\textsuperscript{®}, which demonstrated that BCP and its main component had a better resistance to calcification than commercial patches (****\textit{p}<0.0001) (FIG. 7B)

[106] In vivo studies. Subcutaneous implantation. A set of schematic illustrations of H&E images from three samples: PCU film, Gore-Tex\textsuperscript{®} and CardioCel\textsuperscript{®} Patch, are presented in FIG. 8A-8D. Two polymeric samples, the PCU film and the Gore- Tex\textsuperscript{®} patch, had a layer of tissue capsuled tightly at the interface and both of them kept a relatively intact morphology (FIG.8A (PCU film) VS FIG.8B (Gore-Tex\textsuperscript{®} patch)). For the PCU film, the specimen had delaminated with the adjacent neo-tissue during microtome cutting due to the elastic properties in comparison with the surrounding tissue. There was no cell or tissue growth into the PCU film, whereas cell infiltration occurred in the Gore-Tex\textsuperscript{®} patch. CardioCel\textsuperscript{®} Patch, on the other hand, displayed a different tissue response: first, the patch had a severe degradation. It was hard to observe the intact CardioCel\textsuperscript{®} compared to the control sample (FIG. 8C). Second, cellular nuclei were found in the residue CardioCel\textsuperscript{®} Patch (FIG.8C, bottom, middle) and those were from the adjacent tissues, which indicated the cell infiltration and tissue growth into the patch.

[107] PCU film had no evidence of calcification as indicated by FIG. 8D (top). A high degree of red staining appeared in two commercial patches, indicating calcification in the explant patches and the interface between the encapsulated tissue and the patch sample (red staining is shown as shading in schematic FIGS. 8D (middle) and 8D (bottom)). Little to no calcification was present in most part of the encapsulated tissue.
[108] Subsequently a calcium content assay was conducted and confirmed the histological findings. A significant increase in Ca$^{2+}$ level was found in Gore-Tex® and CardioCel® samples compared to the PCU film, with p<0.0001 (Table 5. and FIG. 8F). Moreover, there was no significant difference in tensile modulus of the PCU film before and after implantation (FIG. 8E).

<p>| Table 5. Calcium Colorimetric Assay Results for the PCU Film, BCP and Commercial Patches |
|---------------------------------------------------------------|---------------------------------------------------------------|</p>
<table>
<thead>
<tr>
<th>Sample</th>
<th>Calcification amount (µg/mg) 16-day In vitro Ca$^{2+}$ Adhesion test</th>
<th>Calcification amount (µg/mg) 8-week In vivo Rat Subcutaneous Test</th>
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</thead>
<tbody>
<tr>
<td>PCU Film</td>
<td>0.022 ± 0.005</td>
<td>0.067 ± 0.006</td>
</tr>
<tr>
<td>BCP</td>
<td>0.039 ± 0.008</td>
<td>-</td>
</tr>
<tr>
<td>Gore-Tex® Patch</td>
<td>1.35 ± 0.19</td>
<td>87.7 ± 4.7</td>
</tr>
<tr>
<td>CardioCel® Patch</td>
<td>1.60 ± 0.24</td>
<td>73.26 ± 8.13</td>
</tr>
</tbody>
</table>

[109] Referring back to FIG. 8A-8D, histological characterization, mechanical property and calcium quantification of the PCU film, Gore-Tex® patch and CardioCel® Patch after in vivo implantation is shown. Sections of the PCU film, Gore-Tex® and CardioCel® had a layer of tissue capsuled at the interface. No cell infiltration or tissue growth within the PCU film but cellular nuclei were found in Gore-Tex® and CardioCel®. CardioCel® had a sign of degradation and cannot obtain an intact morphology (FIGS. 8A-C).

[110] Sections of three samples also displayed the distribution of calcification (red color shown as shading in the schematic illustrations) in tissues and the patch samples. No visible calcification appeared in PCU specimen but a high degree of calcification presented in two commercial specimens (131-133) FIG. 8D. There was no significant change on mechanical
property before and after in vivo implantation (ns, via t-test) (FIG. 8E). Calcium quantification data clearly demonstrated PCU film has a significantly better resistance to calcification in vivo subcutaneous model, with *p<0.0001, compared to the Gore-Tex® patch and CardioCel® Patch (FIG 8F).

[111] Utilizing the biostable and biocompatible polymers as the main components, described herein is a polymer-based, tri-layered patch to mimic the three-layer architecture of native leaflets. The in vitro and in vivo assessment of our BCPs covers two main parts: the long-term mechanical and biological performance.

[112] The mechanical assessment utilizes the cyclic uni-axial tensile tests, flexural bulge tests and suture retention tests for characterization. Tensile test offers a more direct and more economical approach to characterize the mechanical properties. Studies on the uniaxial tensile properties of valve leaflets in the literature have stretched the specimens to break, and recorded the ultimate stress (MPa), the strain-to-failure/ultimate strain (%), as well as calculated the elastic modulus (MPa) using the Equation 1. The ultimate stress and stain-to-failure were acquired beyond the physiological level and unveiled the properties which were not fit the working range; and the one-time tensile stretch cannot reflect the performance at steady state, especially considering the fact that the initial tensile curve behaves more differently from the rest of cyclic curves of viscoelastic materials due to the Mullins’ effect and the preconditioning effects. Tensile modulus, was commonly used to easily quantify an intrinsic elastic property of soft, viscoelastic biomaterials. Note that the stress and strain in the tensile modulus were engineering stress and engineering strain, so the effect of the cross-sectional contraction was not reflected in the tensile modulus.

[113] A 20-time cyclic tensile test for all the samples was conducted. The maximum strain was set as 15% in H/C direction and 40% in V/R direction, corresponding to the physiological level
from systole to diastole. The averaged, post-conditioning curves was picked to do the calculation of tensile modulus to eliminate the influence of the Mullin’s effect and the preconditioning effects. In order to compare BCP with reference tissues and commercial patches, the tensile modulus was calculated at the strain of 15% and 40% for the H/C-direction and V/R-direction, respectively.

[114] The averaged tensile curves and modulus data display that HAV is stiffer than PAV, and PPV is stiffer than PAV. It was also found that the anisotropic behavior and matched mechanical properties at the specific strain range were hardly achieved in commercial patches. Most of them are either too stiff (except for CardioCel® in H direction) or isotropic, compared to the HAV. They are far from the satisfactory material to match the native tissue, from the mechanical view.

[115] BCP, thus, was designed and fabricated using solution casting, lyophilization and electrospinning to replicate the complex, structure-function driven architecture of native leaflets. We hypothesized and demonstrated that a patch with such structure (FIG. 1B) was able to mimic the anisotropic mechanical properties of the native tissue. The aligned PCL fibers were embedded in the PCU film to mimic the fibrosa and the ventricularis. Indeed, the anisotropic properties of the native leaflets come from the orientated dense collagen bundles and elastin network that exist in these two layers. The spongiosa, however, is inherently soft and compliant with a much lower stiffness. Thus, a foam structure made of PCU was designed to mimic the spongiosa. Utilizing the lyophilization, the ice in the frozen-PCU film was removed under the low pressure and the framework inside was kept to maintain its porous structure. This porous structure was demonstrated to confer flexibility and the shock-absorbing properties, as well as offered a relatively lower mechanical stiffness to tailor the BCP. Combining the two fiber-enhanced layers and foam together, the BCP exhibited a tensile modulus of 6.20 ± 1.83 MPa at the strain=15% in the H direction and 1.80 ± 0.21 MPa at the strain=40% in the V direction. Compared to commercial patches, this BCP, for the first time in the literature, to the best of our
knowledge, demonstrated the mimic architectures, anisotropic behaviors and tensile modulus (elasticity) much closer to the human valve leaflets.

[116] The flexural properties of our BCP, heart valve tissues and commercial patches, were also studied using the bulge tests for the first time, in the literature to our best knowledge. Due to the limitation of the pressure transducer and the capacity of the customized syringe pump, the maximum pressure can reach 7.2 kPa (54 mmHg) as a valid, stable level and a frequency of 0.25 Hz allows for specimen inflation. Although it is still far away from the physiological transvalvular peak pressure range (90-120 mmHg) and slower than a normal cardiac cycle frequency (60-100 per minute), under the same conditions, the results from each specimen still demonstrated the various performance on flexural deformation under a quasi-physiological simulation. Three commercial patches displayed a randomly anisotropy performance and higher flexural modulus (4.52-17.58 MPa). Gore-Tex® is made of ePTFE and has no particular design for anisotropic applications. It leads to an isotropic behavior during the tests. While CorMatrix® and CardioCel® are derived from bio tissues and it is reasonable to have some residual fibers in the patch, which provide anisotropy. For our BCP, due to the similar scale of the tensile modulus in-plane X and Y directions, it didn’t display an obvious anisotropic performance in-plane. It also emphasizes the significance to decrease the modulus of the BCPs in V/R direction in order to compare with native tissue level. All of HAV, PAV and BCP have a lower flexural modulus between 0.53-3.55 MPa. This performance is also in line with the trend of tensile modulus data shown in Table 1 and 2, especially the one in C/H direction as shown in FIG. 9. Gore-Tex® and CorMatrix® are much stiffer than native tissues and BCPs. CardioCel®, although much stiffer at strain=40% in V direction, has a compliant performance in H direction and can be compared with HAV and BCP in tensile test and bulge test. From the flexural property view, BCPs offer a good option to be used as alternative patches with a flexural modulus matching the native
leaflets. It also demonstrates the bulge test is valid to acquire flexural properties for further systole-diastole hydrodynamic study and simulation.

[117] Suture retention capability. Punctures and defects are generated during suturing, which may result in mechanical failure through crack propagation. Therefore, the resistance to tear, characterized as SRS and TN-SRS, are essential to evaluate the feasibility of the patches or alternatives. From the results it can be seen the SRS of our BCP and its raw materials (6.25-6.58 N) were in the range of the ones of commercial patches (4.07-8.99 N), which demonstrates that they have a similar capacity of resistance to tearing as the commercial products. It is noted that a number of different suture thread thicknesses and needle types were applied in the clinics, depending on the detailed applications and surgeons’ selection. Some geometrical parameters such as the diameter of the suture, the thickness of the graft wall remain unconstrained by the norm. Thus, TN-SRS was also introduced to evaluate the suture retention capability of the products, normalizing this parameter without impact from the product and thread thicknesses. The TN-SRS of BCP has no significant difference from the ones of commercial patches. And BCP also has a higher toughness than most of commercial patches, which emphasizes its durable nature. To sum up, a series of suture retention tests demonstrated that the BCP has a resistance to tearing similar, even better than the commercial patches, no matter from the SRS, TN-SRS or toughness.

[118] The biological assessment of the BCPs and commercial patches includes the biostability and biocompatibility, in vitro and in vivo. As a designed, polymer-based patch, it is expected to be stable in vivo and the mechanical properties do not alter over time. Published papers reported that the degradation of polyurethane-based materials in vitro and in vivo was attributed to several mechanisms including metal ion-induced accelerated oxidative degradation, hydrolytic degradation and enzymatic degradation. It is demonstrated that oxidative degradation was the more dominant mechanism over other degradations. Thus, a 0.1 M CoCl₂/20% H₂O₂ solution
was applied in this test to accelerate oxidative degradation of the PCUs. The Co²⁺ ions have been demonstrated to rapidly decompose hydrogen peroxide via the Haber-Weiss reaction. Degradation results after 24 days in this solution was shown to correlate to 12 months of in vivo implantation. The modulus of the BCP and PCU film/foam displayed no significant change (NS, One-way ANOVA) on mechanical properties in 30 days in this accelerated oxidization solution. It demonstrated that the BCP has a stable performance which was equivalent to 15 months of in vivo implantation. Even so, a slow oxidative degradation sign was found on the outside surface layer. This finding suggests that the biostability of the BCP, although being comparable to the one of FDA-approved Gore-Tex® patches, may be improved down the road through a surface modification process targeting the resistance to oxidation.

[119] To evaluate biocompatibility, protein adsorption and calcium-ion adhesion are selected to assess BCP and commercial patches’ biological performance in vivo. Protein adsorption is a significant factor to determine the thrombogenicity of an implanted graft. When blood gets in contact with the graft’s surface, protein adsorption occurs first, then leads to more plugs aggregation, eventually provokes the generation of the fibrin network and thrombus formation. Thus, our BCP should aim at reducing their potential for protein adsorption and cut the path of forming thrombin. Bovine serum albumin has been selected in this test since it has a structure similar to human serum albumin (HSA) and the HSA has the highest concentration in human plasma. We performed a BSA protein adsorption test to characterize the blood compatibility of the surfaces of our BCPs and the commercial patches. And the BCP exhibited a low level of protein adsorption compared to three commercial patches. It may be attributed to its smooth PCU film surface without holes or sites, which avoids the plugs deposition and formation.

[120] On the other hand, it is significant to evaluate the resistance to calcification when developing any biomaterial since calcification is the leading reason of failure of bioprosthetic heart valves and grafts. It is a complex phenomenon influenced by a series of mechanical and
biochemical factors. It also limits the durability of synthetic polymer materials used in heart valve devices and blood contact application in general. In vitro Ca\textsuperscript{2+} adhesion tests using a MCP solution to mimics the hydroxyapatite level were performed. A 16-day test exhibits that the BCP and its main component PCU film have a lower level of Ca\textsuperscript{2+} ion accumulation compared to commercial patches. And this trend is also in line with the findings from in vivo subcutaneous tests (FIG. 7D). The BCPs displays a slightly higher mean value than the film, which may be attributed to its porous S-mimic layer embedded between films offering more sites on the side for Ca\textsuperscript{2+} ions accumulation. And it is reasonable to infer the BCP should have a slightly higher calcification level than pristine PCU film but much lower than commercial patches.

[121] An in vivo rat subcutaneous implantation has been conducted to verify the biostability and biocompatibility of PCU film (the main composition of BCPs) and two commercial patches. The former exhibited a stable performance and little/no cell or tissue infuse or grow within the patch. It also exhibited a little-to-no calcification level, better than commercial patches. No obvious mechanical properties degradation after the tests and the tissue generated around the PCU patch were organized and no-calcification. It is a good sign to highlight the feasibility to apply the PCU-based BCP in vivo and expect the positive outcomes.

[122] Compared to three commercial patches, this BCP demonstrated an anisotropic mechanical behavior and mechanical stiffness (6.20 ± 1.83 MPa and 1.80 ± 0.21 MPa in circumferential and radial directions, respectively), which was much closer to the native aortic valve leaflets than any currently available commercial patches. What’s more, our BCPs also showed an excellent durability in an in vitro accelerated oxidization solution and displayed an excellent biocompatibility with an in vitro lower protein adsorption level and a lower calcium adhesion level. In vivo rat subcutaneous tests confirmed its main composition, PCU’s mechanical biostability and superior resistance to inflammation and calcification, compared to the commercial patches.
[123] The native-like performance of the BCP avoids patch failure and degeneration, which are related to the inadequate mechanical properties. It is biostable, and does not rely on uncontrolled polymer degradation and tissue formation. The biomimetic patch also exhibits a low protein adsorption and low Ca$^{2+}$ adhesion, avoiding a high risk of thrombogenicity and calcification. In some embodiments, fiber meshes can be fabricated by various biocompatible polymers, to optimize the anisotropic mechanical performance.

[124] In some embodiments, the biostability and biocompatibility is optimized through adding the surface layer on the current version, for example, Parylene C can be evenly coated on the patch through chemical vapor deposition.

[125] The biomimetic patch is scalable. For example, at a lab scale, this version of the patch is processed through solution casting, electrospinning and lyophilization. A multiple technology combination provides flexible tuning methods for optimization. At an industrial scale, this tri-layer composite can be fabricated via a non-expensive and scalable multi-layer co-extrusion technology. This green, non-solvent involved method provides a better reproducibility and lower costs of production. It provides a feasible path to commercialize this polymeric patch to improve the durability and quality of the valve repair, and decrease the number of reoperations and complications. In some embodiments, the surface morphology is further processed to create the “corrugations” structure to mimic the native leaflet’s surface morphology. This structure plays an important role and accounts for the native collagen fiber’s mechanical behavior during valve closing.

[126] While the disclosure has been illustrated and described in detail in the drawings and foregoing description, such illustration and description are to be considered illustrative or exemplary and not restrictive. The disclosure is not limited to the disclosed embodiments. Variations to the disclosed embodiments and/or implementations can be understood and effected
by those skilled in the art in practicing the claimed disclosure, from a study of the drawings, the disclosure and the appended claims.
CLAIMS

What is claimed is

1. A biomimetic biomaterial patch configured to mimic native heart valve tissue, the patch comprising a composite body including:
   a polymeric Fibrosa-mimic ("F-mimic") layer;
   a polymeric Spongosia-mimic ("S-mimic") layer; and
   a polymeric Ventricularis-mimic ("V-mimic") layer.

2. The biomimetic biomaterial patch of claim 1, wherein the F-mimic layer and the V-mimic layer of the composite body are anisotropic and the S-mimic layer is a shock absorbing layer.

3. The biomimetic biomaterial patch of claim 1, wherein the F-mimic layer is formed of polycarbonate polyurethane (PCU) film having embedded polycaprolactone (PCL) fibers therein.

4. The biomimetic biomaterial of claim 1 and 3, wherein the V-mimic layer is formed of polycarbonate polyurethane (PCU) film having embedded polycaprolactone (PCL) fibers therein.

5. The biomimetic biomaterial patch of claims 3 and 4, wherein at least one of the F-mimic and the V-mimic layers are each made of polycarbonate polyurethane (PCU) film having embedded aligned polycaprolactone (PCL) fibers therein.

6. The biomimetic biomaterial patch of claim 5, wherein the PCL fibers are electrospun.
7. The biomimetic biomaterial patch of claim 6, wherein the electrospun fibers exhibit a highly oriented distribution.

8. The biomimetic biomaterial patch of claim 7, wherein the electrospun fibers exhibit anisotropic performance during cyclic tensile tests compared to random electrospun PCL fibers.

9. The biomimetic biomaterial patch of claim 7, wherein the F-mimic and V-mimic layers demonstrate anisotropic behavior in first and second directions, the first and second directions being different directions.

10. The biomimetic biomaterial patch of claim 9, wherein the first direction is the H direction and the second direction is the V direction.

11. The biomimetic biomaterial patch of claim 5, wherein the tensile modulus are from about 25 to about 40 MPa at the strain in the H direction and about 1 to 3 MPa at the strain in the V direction.

12. The biomimetic biomaterial patch of claim 1, wherein the S-mimic layer is formed from PCU foam.

13. The biomimetic biomaterial patch of claim 1, wherein the F-mimic layer is coated on one surface of the S-mimic layer and the V-mimic layer is coated on the opposing surface of the S-mimic layer to form a composite biomimetic patch structure.

14. The biomimetic biomaterial patch of claim 1, wherein the biomimetic patch exhibits anisotropic mechanical behavior similar to native human valve leaflets.
15. The biomimetic biomaterial patch of claim 1, wherein the biomaterial exhibits a tensile modulus of about 4 to 8 MPa at the strain in a first direction.

16. The biomimetic biomaterial patch of claim 1, wherein the biomaterial exhibits a tensile modulus of about 1.5 to 2 MPa at the strain in a second direction.

17. The biomimetic biomaterial patch of claim 1, wherein the S-mimic layer is made of PCU foam and the F-mimic and the V-mimic layers are each made of polycarbonate polyurethane (PCU) film having embedded electrospun, aligned polycaprolactone (PCL) fibers therein.

18. The biomimetic biomaterial patch of claim 1, wherein the patch is entirely made of polymeric material.

19. The biomimetic biomaterial patch of claim 1, wherein the patch is limited to three layers.

20. The biomimetic biomaterial patch of claim 1, wherein the patch is mechanically stable for clinical use.

21. A stable biomimetic biomaterial comprising

   a first layer comprising polycarbonate polyurethane (PCU) film embedded with aligned polycaprolactone (PCL) fibers,

   a second layer comprising PCU foam, and
a third layer comprising polycarbonate polyurethane (PCU) film embedded with aligned polycaprolactone (PCL) fibers, wherein the layers form a composite structure.

22. The stable biomimetic biomaterial of claim 21, wherein the composite structure lacks animal-derived tissue.

23. The stable biomimetic biomaterial of claim 21, wherein the patch exhibits a low protein adsorption.

24. The stable biomimetic biomaterial of claim 21, wherein the patch exhibits low Ca\textsuperscript{2+} adhesion.

25. The stable biomimetic biomaterial of claim 21, further comprising a surface layer disposed at least one surface of the composite structure.

26. The stable biomimetic biomaterial of claim 25, wherein the surface layer comprises Parylene C.

27. The stable biomimetic biomaterial of claim 21, at least one surface of the composite structure includes a corrugated structure to mimic morphology of the native heart leaflet surface.

28. The stable biomimetic biomaterial of claim 21, wherein the biomaterial exhibits anisotropic mechanical behavior similar to native human valve leaflets.

29. The stable biomimetic biomaterial of claim 21, wherein the biomaterial exhibits a tensile modulus of about 4 to 8 MPa at the strain in a first direction.
30. The stable biomimetic biomaterial of claim 29, wherein the biomaterial exhibits a tensile modulus of about 1.5 to 2 MPa at the strain in a second direction.

31. The stable biomimetic biomaterial of claim 29, wherein the material is in the form of a heart patch repairing material.

32. An implantable prosthetic heart valve comprising:

   a plurality of leaflets, each leaflet formed from a polymeric biomaterial, wherein the polymeric biomaterial is a composite body including a polymeric Spongiosa-mimic (“S-mimic”) layer and at least one polymeric layer selected from the group consisting of: a polymeric Fibrosa-mimic (“F-mimic”) layer; and a polymeric Ventricularis-mimic (“V-mimic”) layer, or a combination thereof.

33. The prosthetic heart valve of claim 32, wherein the F-mimic layer and the V-mimic layer are anisotropic and the S-mimic layer is a shock absorbing layer each leaflet.

34. The prosthetic heart valve of claim 32 or 33, wherein the F-mimic layer is formed of polycarbonate polyurethane (PCU) film having embedded polycaprolactone (PCL) fibers therein.

35. The prosthetic heart valve of claim 34, wherein the embedded PCL fibers are aligned fibers.

36. The prosthetic heart valve of claim 34 and 35, wherein the PCL fibers are from electrospun.
37. The prosthetic heart valve of claims 32, wherein the prosthetic comprises a tri-layered composite body including:
   a polymeric Fibrosa-mimic (“F-mimic”) layer;
   a polymeric Spongiosa-mimic (“S-mimic”) layer; and
   a polymeric Ventricularis-mimic (“V-mimic”) layer.

38. The prosthetic heart valve of claim 37, wherein at least one of the F-mimic and the V-mimic layers are each made of polycarbonate polyurethane (PCU) film having embedded aligned polycaprolactone (PCL) fibers therein.

39. The prosthetic heart valve of claim 38, wherein the PCL fibers are electrospun.

40. The prosthetic heart valve of claim 39, wherein the electrospun fibers exhibit a highly oriented distribution.

41. The prosthetic heart valve of claim 39, wherein the electrospun fibers exhibit anisotropic performance during cyclic tensile tests compared to random electrospun PCL fibers.

42. The prosthetic heart valve of claim 41, wherein the F-mimic and V-mimic layers demonstrate anisotropic behavior in first and second directions, the first and second directions being different directions.

43. The prosthetic heart valve of claim 42, wherein the first direction is the H direction and the second direction is the V direction.
44. The prosthetic heart valve of claim 43, wherein the tensile modulus are from about 25 to about 40 MPa at the strain in the H direction and about 1 to 3 MPa at the strain in the V direction.

45. The prosthetic heart valve of claim 37, wherein the S-mimic layer is formed from PCU foam.

46. The prosthetic heart valve of claim 37, wherein the F-mimic layer is coated on one surface of the S-mimic layer and the V-mimic layer is coated on the opposing surface of the S-mimic layer to form a composite biomimetic patch structure.

47. The prosthetic heart valve of claim 37, wherein the composite body exhibits anisotropic mechanical behavior similar to native human valve leaflets.

48. The prosthetic heart valve of claim 37, wherein the composite body exhibits a tensile modulus of about 4 to 8 MPa at the strain in a first direction.

49. The prosthetic heart valve of claim 37, wherein the composite body exhibits a tensile modulus of about 1.5 to 2 MPa at the strain in a second direction.

50. The prosthetic heart valve of claim 36, wherein the S-mimic layer is made of PCU foam and the F-mimic and the V-mimic layers are each made of polycarbonate polyurethane (PCU) film having embedded electrospun, aligned polycaprolactone (PCL) fibers therein.

51. The prosthetic heart valve of claim 36, wherein the composite body is entirely made of polymeric material.
52. The prosthetic heart valve of claim 36, wherein the composite body includes from two to five polymeric layers.

53. The prosthetic heart valve of claim 36, wherein the composite body lacks animal-derived tissue.

54. The prosthetic heart valve of claim 36, wherein the prosthetic heart valve is an aortic valve, mitral valve, or a tricuspid valve.

55. A method of treating a heart defect comprising the steps of:

   providing a biomimetic biomaterial patch according to any one of claims 1 - 20,

   placing the biomimetic biomaterial patch on an uninflated distal balloon,

   placing the biomimetic biomaterial patch and the balloon distally of the defective opening,

   inflating the balloon,

   moving the balloon and the patch on the balloon firmly against the defective opening,

   permitting the patch to contact to the heart defect, then

   deflating and removing the balloon.

56. The method of claim 55, wherein the biomimetic biomaterial patch endothelializes to the defect.
57. The method of claim 55, wherein the biomimetic biomaterial patch occludes the heart defect.

58. The method of claim 55, wherein the biomimetic biomaterial patch is percutaneously delivered to the heart defect.

59. The method of claim 55, wherein the balloon is mounted on a delivery catheter.

60. The method of claim 55, wherein the biomimetic biomaterial patch has a shape that matches the shape of the cardiac site to be repaired.

61. A method of replacing a heart valve in a subject, comprising the steps of:

   inserting a distal end portion of a delivery sheath into a portion of a heart of a subject, the delivery sheath having a prosthetic heart valve according to any one of claims 32-54 disposed within a lumen of the delivery sheath,
   moving the prosthetic heart valve distally out of the delivery sheath; and
   positioning the prosthetic heart valve within the heart.

62. The method of claim 61, wherein the method is a method for treating the subject for aortic stenosis, mitral valve stenosis, regurgitation, or tricuspid valve regurgitation.

63. A stable biomimetic biomaterial comprising

   a polycarbonate polyurethane (PCU) foam layer, and
a plurality of aligned polypropylene fibers embedded in the PCU layer, such that
the plurality of aligned polypropylene fibers are spaced from each other.

64. The stable biomimetic biomaterial of claim 63, wherein the polypropylene fibers are
sutures.

65. The stable biomimetic biomaterial of claim 63 or 64, wherein the plurality of
propylene fibers includes fibers having different sizes.

66. The stable biomimetic biomaterial of claim 65, wherein the sizes of the
polypropylene fibers are selected from the group consisting of 6-0, 7-0, and 8-0.

67. The stable biomimetic biomaterial of claim 63, wherein the plurality of
polypropylene fibers includes up to 4 fibers.

68. The stable biomimetic biomaterial of claim 63, wherein the biomaterial exhibits a
tensile modulus in a C/H direction of about 8 to about 16 MPa.

69. The stable biomimetic biomaterial of claim 63, wherein the biomaterial exhibits a
tensile modulus in a R/V direction of about 0.57 to about 0.68 MPa.
Two Suture-Foams Comparison

- Suture 7-0-foam 15%-H
- Suture 7-0-foam 40%-V
- Suture 6-0-foam 15%-H
- Suture 6-0-foam 40%-V
- HAV-C
- HAV-R

FIG. 1F
Human vs Porcine Leaflets
Stress-Strain Average Curves

FIG. 2C
CardioCel Patch Stress-Strain Average Curves

- Average curve 15%(H)
- Average curve 15%(V)
- Average curve 40%(H)
- Average curve 40%(V)

FIG. 2F

C/H Direction
@strain=15%

R/V Direction
@strain=40%

Tensile Modulus (MPa)

FIG. 2G
FIG. 4A

FIG. 4B
FIG. 4C

Human aortic valve leaflet flexural deformation

FIG. 4D
**FIG. 6A**

PCU Film/ Foam & BCP biostability

vs

Commercial Patch biostability

- ○ BCP (H&V)
- ● PCU film
- □ PCU foam
- △ Gore-Tex
- ▲ CardioCel Patch
- ○ CorMatrix Patch

**FIG. 6B**

PCU film 30-day Biostability Test

One-way ANOVA, ns
**FIG. 7A**

- BCP: 5.1±1.1
- Gore-Tex: 6.9±1.3
- CoMatrix: 91±7.5
- CardioCel: 53.8±24.1

**FIG. 7B**

- PCU film: 0.021±0.004
- BCP: 0.039±0.006
- Gore-Tex: 1.35±0.16
- CardioCel: 1.6±0.2
FIG. 9
A. CLASSIFICATION OF SUBJECT MATTER
IPC - A61L 27/18; A61F 2/24; A61B 17/12; C08G 18/42 (2021.01)
CPC - A61F 2/2427; C08G 18/42; A61L 27/18; Y10S 623/924

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED
Minimum documentation searched (classification system followed by classification symbols)
See Search History document

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched
See Search History document

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
See Search History document

C. DOCUMENTS CONSIDERED TO BE RELEVANT

<table>
<thead>
<tr>
<th>Category*</th>
<th>Citation of document, with indication, where appropriate, of the relevant passages</th>
<th>Relevant to claim No.</th>
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<tr>
<td>Y</td>
<td>US 2010/0318108 A1 (DATTA et al.) 16 December 2010 (16.12.2010); para [0042], [0045], [0078], [0096], [0105], [0110], [0155], [0157], [0165], [0169], [0172], [0205], [0227], [0229], [0325], [0387]</td>
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<td>X</td>
<td>US 2002/0123599 A1 (LEVY et al.) 05 September 2002 (05.09.2002); para [0004], [0027], [0086], [0112]-[0113], [0119]</td>
<td>63-69</td>
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<tr>
<td>A</td>
<td>CHEN Q.-Z. et al., &quot;Characterisation of a soft elastomer poly(glycerol sebacate) designed to match the mechanical properties of myocardial tissue&quot;, Biomaterials, 2008, volume 29, issue 1, pp. 47-57, retrieved from the Internet: &lt; DOI: 10.1016/j.biomaterials.2007.09.010 &gt;; see entire document</td>
<td>26</td>
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* Further documents are listed in the continuation of Box C. See patent family annex.

Date of the actual completion of the international search
09 February 2021

Date of mailing of the international search report
MAR 15 2021

Name and mailing address of the ISA/US
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Facsimile No. 571-273-8300

Authorized officer
Lee Young

Telephone No. PCT Helpdesk: 571-272-4300

Form PCT/ISA/210 (second sheet) (July 2019)
INTERNATIONAL SEARCH REPORT

Box No. II  Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
   because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.:
   because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☒ Claims Nos.: 4-11, 36 and 50-62
   because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box No. III  Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying additional fees, this Authority did not invite payment of additional fees.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest  ☐ The additional search fees were accompanied by the applicant’s protest and, where applicable, the payment of a protest fee.

☐ The additional search fees were accompanied by the applicant’s protest but the applicable protest fee was not paid within the time limit specified in the invitation.

☐ No protest accompanied the payment of additional search fees.
<table>
<thead>
<tr>
<th>Category</th>
<th>Citation of document, with indication, where appropriate, of the relevant passages</th>
<th>Relevant to claim No.</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>WO 2006/003763 A2 (THE UNIVERSITY COURT OF THE UNIVERSITY OF GLASGOW) 05 January 2006 (05.01.2006); see entire document</td>
<td>1-3, 12-35, 37-49, 63-69</td>
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