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# Emerging Trends in Heart Valve Engineering: Part I. Solutions for Future

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**Abstract**—As the first section of a multi-part review series, this section provides an overview of the ongoing research and development aimed at fabricating novel heart valve replacements beyond what is currently available for patients. Here we discuss heart valve replacement options that involve a biological component or process for creation, either *in vitro* or *in vivo* (tissue-engineered heart valves), and heart valves that are fabricated from polymeric material that are considered permanent inert materials that may suffice for adults where growth is not required. Polymeric materials provide opportunities for cost-effective heart valves that can be more easily manufactured and can be easily integrated with artificial heart and ventricular assist device technologies. Tissue engineered heart valves show promise as a regenerative patient specific model that could be the future of all valve replacement. Because tissue-engineered heart valves depend on cells for their creation, understanding how cells sense and respond to chemical and physical stimuli in their microenvironment is critical and therefore, is also reviewed.

**Keywords**—Heart valve engineering, Tissue engineered heart valves, Polymeric heart valves.

## INTRODUCTION

Valvular heart disease is, particularly as the population ages, an increasingly common cause of cardiovascular disease in the United States and is equally impactful around the globe. In the developing world, rheumatic valve disease causes 492,042 deaths annually, comparable to all cardiovascular disease-related deaths in the U.S.<sup>23</sup> This burden of disease leads to over 300,000 heart valve replacement surgeries each year worldwide.<sup>107</sup> There is currently no medical treatment for a dysfunctional heart valve, and repair or replacement of a dysfunctional valve remains as the only option that markedly reduces the morbidity and mortality associated with heart valve disease. It is anticipated that the number of patients requiring valve replacement worldwide will triple by 2050,<sup>107</sup> leading some to describe heart valve disease as “the next cardiac epidemic”.<sup>37</sup>

Heart valve engineering is a branch of biomedical engineering focused on the research and development of devices to replace or repair a diseased heart valve. When approaching heart valve replacement options, there are two main approaches to fabricate valves: biological and non-biological. Occasionally, both approaches are combined in hybrid technologies. The biological approach aims to replicate a native heart

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valve by combining living cells (valvular cells, stem cells, *etc.*) with a biocompatible scaffold (biopolymer, cell-produced extracellular matrix, synthetic polymer, *etc.*). Non-biological options are those without live cellular/tissue elements such polymeric, bioprosthetic and mechanical valves. While the form and function of the biological valve, from the stand-point of its living cells, must closely mimic the healthy native valve; this requirement is not stringent for the non-biological option where function, durability, and hemocompatibility are the primary factors that govern the engineering of these devices. Utilizing biological approaches, which depend crucially on the micro (mechanical) environment experienced by the cells, tissues are grown to the shape of a valve *in vitro*, often in a bioreactor applying exogenous chemical stimulation or mechanical loading, and then implanted in the body. The engineered tissue or the scaffold may also be combined with inert materials as they may provide improved durability over the current use of chemically treated animal tissue (porcine valves and valves fabricated from bovine pericardium), which eventually fail due to calcification and/or insufficient recellularization.<sup>51,89,95</sup> Heart valve tissue engineering aims for a permanent solution to the large number of congenital valvular abnormalities in pediatric patients and young adults for whom currently available replacement valves are poorly suited.<sup>21,87</sup> The main motivation for these efforts is to biologically engineer durable living replacement heart valves with the capacities to grow (pediatric patients) and/or repair (adult patients), which requires the engineered tissue that is implanted to appropriately remodel. In this part of this four part review, we discuss about the current state of the art of heart valve engineering concepts with an emphasis on tissue-engineered and polymeric valves that are not currently available for patients but are believed to be the trend setters for future heart valves. Part II of the series reviews the novel and standard technologies for aortic valves replacement. Part III of the series focused on the repair and replacement options for the mitral valve. Finally, part IV is a focused review of advanced computational modeling of and experimental testing of heart valves.

## TISSUE ENGINEERED HEART VALVES (TEHVS)

One of the primary goals of TEHVs is to mimic the form and function of the native heart valve tissue. To understand this task, let us first briefly outline the relevant biomechanical characteristics of the native leaflet's form-function relationship that needs to be emulated. The healthy native leaflets can open and close a few billion times without failure, while withstanding the

stresses generated by a trans-valvular pressure of the order of 100 mmHg. There are many unique aspects of nature's design that make this feat achievable. All native leaflet tissue is organized in three layers with a total thickness less than a millimeter. For the aortic leaflets for instance, the layer facing the ventricular side is called the ventricularis. The middle layer is called spongiosa, while the layer facing the aorta is called fibrosa. Each of these layers is made of an extra-cellular matrix composed of biological materials such as collagen, elastin, Glycosaminoglycans (GAGs) such as Hyaluronan, and other proteoglycans *etc.* It is believed that the three layer structure is what makes leaflets incredibly strong, yet highly flexible. Flexibility, i.e., low bending stiffness, is important to ensure the leaflets open easily and maximize the flow area. The strength and elasticity characteristics make the leaflets stretch, just enough, as the leaflets coapt and close, in a manner that makes valve closure a smooth affair (i.e., no "water hammer" effect) while ensuring no regurgitation. A closer look at the three layers have shown that the ventricularis is primarily made of collagen and elastic fibers arranged in a dense network with endothelial cells lining the edge. The fibrosa on the other hand consists of dense collagen fibers alone with endothelial cells lining the edge. The spongiosa in the middle is a gel like layer without fibers and consists mainly of proteoglycans, hydrated GAGs, and interstitial cells. Some interstitial cells do exist also in the ventricularis and fibrosa layers near the spongiosa (i.e., there is no clear demarcation line distinguishing the three layers, and the transition between layers is smooth). However, roughly the fibrosa constitutes 41% and ventricularis constitutes 29% of the thickness.<sup>88</sup> While this is the general organization of the leaflet tissue of aortic valves, the pattern is similar for the other native valves. An organization of this sort allows for fine tuning (or remodeling) of gross mechanical properties unique to the valve environment, as well as in response to pathological conditions (such as congestive heart failure, or stress). Further, it has been recently hypothesized that the gross mechanical properties can vary even during the cardiac cycle,<sup>57,58</sup> and that these layers are in a state of pre-strain<sup>78</sup> to ensure that the valve leaflets are operating in their most "effective" portions of their respective constitutive properties. This allows the leaflets to practically program such that a desired stress within the leaflets may be generated for a given strain (which is dictated more by the geometric conditions of the valve).

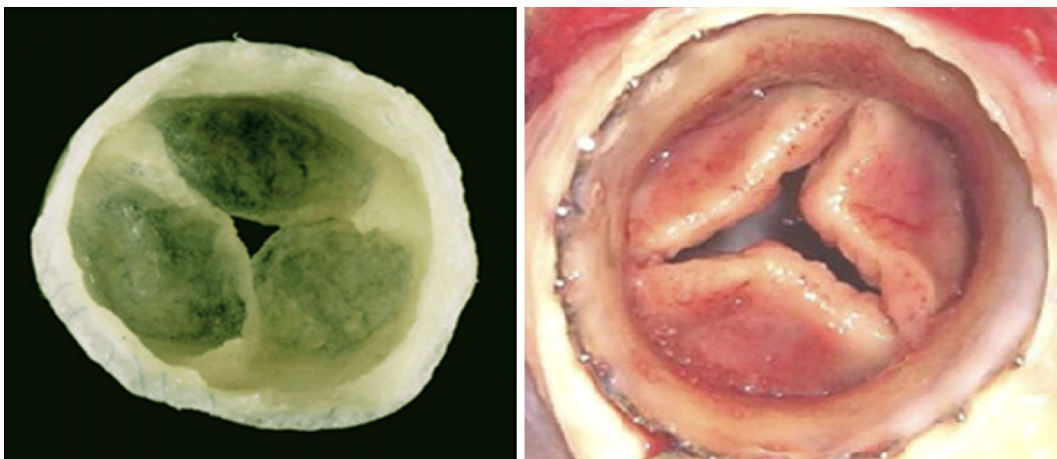
In general, the overall biomechanical characteristics of the valve leaflets define a highly non-linear stress-strain behavior with complex viscoelasticity and axial coupling to allow for large deformations.<sup>42,86</sup> Almost all of the "load bearing" occurs within the collagen fibers. It is the orientation of these collagen fibers

within the leaflets, combined with how they stretch and rotate as the leaflet deforms, that generates such a complex yet highly tuned stress-strain behavior. Organization of fibers is location specific even within a leaflet. For instance the coaptation region, which defines the area where leaflets touch each other when the valve is closed, experiences no trans-valvular pressure loading. The loading in this region including the commissures is predominantly uni-axial. Closer to the commissural attachment point where the leaflets connect to the aorta, the tissue structure and fiber orientations resemble that of a tendon where fibers are strongly aligned in the direction of loading and exist in a state where the transition from uncrimped to crimped states can occur at low strains.

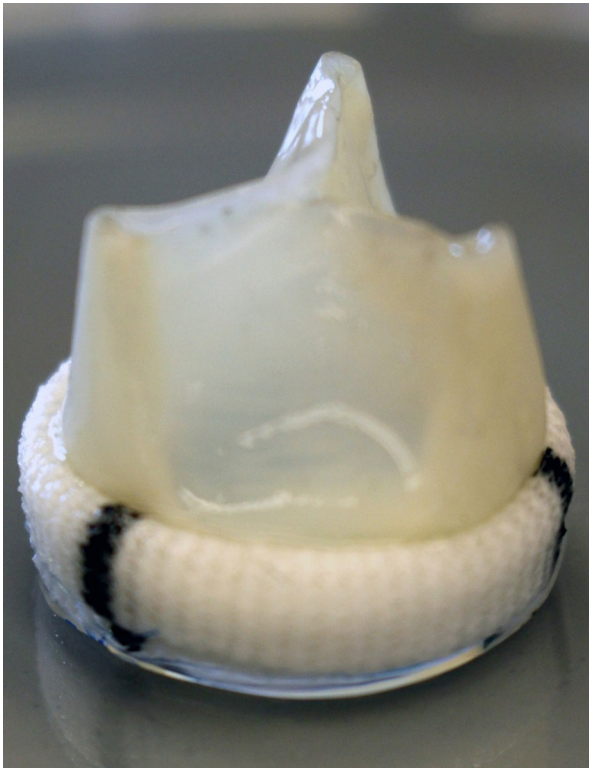
There have already been three decades of researching TEHVs to replace diseased natural valves.<sup>20,65,75,76,87,98,101</sup> Thus far, a few groups have conducted *in vivo* studies to test these valves, mostly in the pulmonary position. The current state of the research is primarily based on two distinct approaches. In the first approach, the traditional *in vitro* grown, tissue-engineered valve is typically created by seeding cells (e.g., patient-specific, progenitor/stem) in a synthetic scaffold or a decellularized donor valve, and then the cell-seeded biomaterial is biochemically and biomechanically conditioned in a bioreactor to generate a mechanically competent tissue for implantation.<sup>87</sup> The second approach relies on the natural regenerative potential of the body to populate the unseeded scaffold after implantation. The latter includes cell-free scaffolds that recruit circulating endogenous (progenitor) cells from the blood stream and are intended to transform gradually into a living structure. This strategy offers an off-the-shelf availability; however,

controlling cell recruitment and tissue formation (particularly thickness of the tissue and ECM components) inside the body are the main challenges to this approach.<sup>18</sup>

The early efforts in generating TEHVs mainly focused on seeding cells into synthetic scaffolds such as polyglycolic acid (PGA) meshes and then evaluating their performance *in vivo*. While the results of these early animal studies were promising, the resulting leaflet tissues were found to be either thicker or stiffer than native valves.<sup>92,93</sup> To overcome this concern, a combination of PGA and poly-4-hydroxybutyrate (P4HB) was used in later TEHV development.<sup>51</sup> After 20 weeks of implantation at the pulmonary position, it was observed that the TEHV showed mechanical behavior and trilaminar structure similar to that of a native valve (Fig. 1). However, the long-term fate (after 20 weeks) of these early TEHVs was unknown.<sup>51</sup> More recently, a minimally invasive version of this TEHV has been implanted in animals with favorable results (Fig. 1; right), although later time points showed leaflet thickening.<sup>89</sup> Sutherland *et al.* reported a combination use of PGA and poly-L-lactic acid (PLLA) in their scaffold, which was implanted using autologous bone-marrow derived mesenchymal stem cells in the pulmonary position for up to 8 months.<sup>95</sup> Although the results showed *in vivo* remodeling with a structure comparable to native valves, the leaflet retraction negatively impacted their long-term functionality. Recently, Dijkman *et al.*<sup>38</sup> proposed to decellularize the *in vitro* cultured TEHV prior to implantation, creating homologous off-the-shelf available TEHVs. These valves rapidly repopulated *in vivo* with host derived cells, both in senescent non-human primate studies<sup>103</sup> as well as in an ovine



**FIGURE 1.** (Left) One of the most successful autologous tissue-engineered valves that resembled normal heart valves in microstructure, mechanical properties, and extracellular matrix formation. Figure from Hoerstrup *et al.*<sup>51</sup>; (Right) Minimally invasive implanted tissue-engineered valve. Figure from Schmidt *et al.*<sup>89</sup>



**FIGURE 2.** A completely biological valve developed by Tranquillo group. Unlike all previous TEHV, it does not entail a design based on native valve anatomy (i.e., an aortic root with attached leaflets).

model.<sup>39</sup> No leaflet thickening was observed, but leaflet retraction still compromised long-term functionality of these valves.

In another effort to implement the use of natural scaffolds, Syedain *et al.*<sup>97</sup> developed a TEHV from a decellularized tube of engineered tissue mounted on a frame with three struts, which upon back-pressure caused the tube to collapse into three coapting “leaflets” (Fig. 2). This concept, pioneered by Cox *et al.*,<sup>32</sup> is embodied in several commercial valves; however, those valves employ sewn glutaraldehyde-treated pericardial tissue, which does not become recellularized and, thus, have a lifetime limited to 15–20 years as is typical of bioprosthetic valves. The tissue used for this tubular TEHV is completely biological, fabricated from dermal fibroblasts dispersed within a fibrin gel, compacted into a circumferentially aligned tube on a mandrel, and matured using a bioreactor system that applies cyclic distension. Following decellularization, the resulting matrix possessed tensile mechanical properties, mechanical anisotropy, and collagen content that were comparable to native pulmonary valve leaflets. When mounted on a custom frame and tested within a pulse duplicator system, the tubular TEHV displayed excellent function under both aortic and

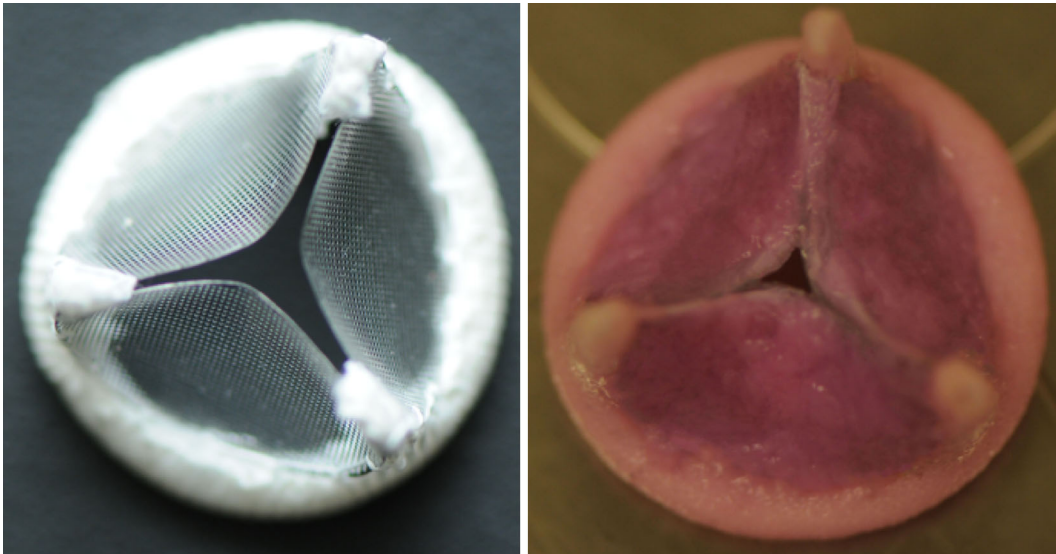
pulmonary conditions, with minimal regurgitant fractions (<5%) and transvalvular pressure gradients at peak systole (<3 mmHg), as well as high effective orifice areas. Short-term fatigue tests of one million cycles with pulmonary pressure gradients were conducted without significant change in mechanical properties and no observable macroscopic matrix deterioration. This matrix exhibited favorable remodeling, including host cell recellularization and spontaneous endothelialization, once implanted into the sheep femoral artery as an interpositional vascular graft.<sup>96</sup> It thus presents potential for tissue durability without the need for anti-coagulation therapy. Further studies are underway to assess this potential.

Hybrid scaffolds containing multiple biological molecules have been widely used to develop tissue-engineered organs. In a fundamentally different type of hybrid, Alavi and Kheradvar<sup>3</sup> developed a scaffold with a combination of a natural biological material (collagen) and a non-degradable metal mesh material made of Nitinol. Nitinol, an alloy of Nickel and Titanium, is currently used for several cardiovascular applications including peripheral vascular stents and transcatheter heart valves. It has been shown to be biocompatible and exhibit superior strain controlled fatigue performances to those of other materials.<sup>67</sup> Through this approach, hybrid heart valves were developed with leaflets made of a thin Nitinol mesh core tightly enclosed by multiple layers of smooth muscle cells, fibroblasts/myofibroblasts, and endothelial cells in a similar fashion to a native valve (Fig. 3). After addition of the cells to the scaffold there is a similar pattern of cell type and densities when compared to native valves, and therefore comparable mechanical properties. The leaflets’ thickness is up to 600  $\mu\text{m}$  and after culture, does not increase in size due to the culture method.

Based on their hypothesis, a scaffold containing a very thin super-elastic Nitinol sheet can serve as the primary load-bearing component of the hybrid leaflet, and should preserve the structural integrity of the valve when subjected to high cardiac pressures, particularly in the left ventricle. Using the patient’s own cells for the culture will turn this valve into a patient-specific one with the capacity for self-regeneration and the potential for repair and remodel.<sup>73</sup> This valve has been successfully tested *in vitro*,<sup>2–4</sup> although pre-clinical and clinical studies will be required for a full assessment of the valve’s function and durability.

## REGULATION OF THE MICROENVIRONMENT

A challenge faced by all the tissue engineering strategies for heart valves is to identify cell sources,



**FIGURE 3.** A hybrid valve developed by Alavi and Kheradvar; the left panel shows the trileaflet Nitinol scaffold prior to cell seeding. The right panel shows the same trileaflet scaffold 8 weeks after cell seeding. The thin (25  $\mu\text{m}$ ) Nitinol mesh is fully enclosed within the three layers of smooth muscle, fibroblast/myofibroblast and endothelial cells. This valve has shown promising results in *in vitro* studies.

scaffolds, and *in vitro* bioreactor conditions that appropriately guide cell growth, differentiation, and synthesis of the extracellular matrix to ultimately produce functional, durable valve tissue. Progress towards this goal should be guided by knowledge of how the native valve microenvironment controls cell fate and function and tissue remodeling.<sup>27,46,109</sup> Valve disease recapitulates many aspects of valve development,<sup>50,90</sup> and therefore knowledge of the role of the microenvironment in valve disease can be instructive for progenitor cell-based valve tissue engineering, particularly in light of the similarities between valvular interstitial cells (VICs) and mesenchymal stem cells (MSCs).<sup>28</sup>

The VIC microenvironment is in part defined by the valvular extracellular matrix (ECM) and its mechanical and biochemical properties, to which VICs are sensitive. For example, ECM elasticity regulates *in vitro* pathological differentiation of VICs to myofibroblasts and osteoblasts in a manner similar to bone marrow-derived MSCs<sup>41</sup>; VICs are primarily fibroblastic on soft (<10 kPa elastic modulus) substrates; preferentially differentiate to osteoblasts on intermediate (~15–20 kPa) substrates; and differentiate to myofibroblasts with  $\alpha$ -smooth muscle actin-positive stress fibers on stiffer (>25 kPa) substrates.<sup>25,56,72,108</sup> VIC-ECM protein interactions also regulate VIC myofibroblast differentiation and contraction. Fibronectin,<sup>33</sup> fibrin,<sup>79</sup> elastin,<sup>33</sup> and the fibronectin adhesion peptide RGDS (Arg-Gly-Asp-Ser)<sup>48</sup> generally promote myofibroblast differentiation. Furthermore, type I collagen,<sup>33,79</sup> along with the laminin peptides YIGSR (Tyr-Ile-Gly-Ser-Arg) and collagen peptide DGEA (Asp-Gly-Glu-Ala)<sup>48</sup> suppress myofibroblast differentiation. It should be

noted, however, that presentation of multiple peptides simultaneously, or presentation of peptides on compliant substrates, can yield different responses due to synergism.<sup>45</sup> Growth factors (e.g., transforming growth factor (TGF)- $\beta$ 1) and cyclic stretch also define the microenvironment. Both can elicit responses associated not only with valve disease, but also development, repair, and regeneration, including increased matrix synthesis and remodeling,<sup>6–8</sup> and VIC activation to the secretory myofibroblast phenotype.<sup>6,66,69</sup>

All in all, there is significant interaction between various types of microenvironmental cues, which profoundly impact cell responses. In the context of VIC biology, TGF- $\beta$ 1 and cyclic strain synergistically act to enhance VIC myofibroblast differentiation and collagen synthesis.<sup>66,69,96</sup> VIC response to cyclic strain is ECM protein-dependent,<sup>69</sup> similar to the MSC response to ECM elasticity.<sup>26</sup> To develop a means of delivering customized combinations of microenvironmental cues to the cells within a TEHV, there is growing interest in the use of scaffolds based on synthetic hydrogel materials. This class of scaffold materials can be mixed with cells before polymerization, poured into a specifically shaped mold, then quickly cross-linked *in situ* using various mechanisms (i.e., thermal, chemical, light). The resulting cross-linked hydrogel will have encapsulated cells evenly distributed throughout its interior. Many different hydrogels and photoinitiators are in use, but hydrogels made from polyethylene glycol (PEG) permit additional modifications that imbue the hydrogel with highly specific biofunctionality. Peptides, proteins, or polysaccharides can be incorporated into the polymer backbone or

grafted into the hydrogel network during photopolymerization.<sup>60,62</sup> Hydrogels have finely tunable material properties and swelling behavior based on the concentration and molecular weight (MW) of the hydrophilic polymer.<sup>40</sup> As noted above, heart valve cells can vary their phenotypes when they are cultured on substrates of different stiffness. In a study of mitral valve cells cultured atop either soft or stiff PEG-based hydrogels, the expression of smooth muscle alpha-actin, prolyl-4-hydroxylase, and heat shock protein 47 varied between the two different substrates. The magnitude of the response, however, was also influenced by the original environment of the cells within the mitral valve, and the age of the animal from which the valves were harvested.<sup>94</sup>

The ability to integrate regionally varying material properties and biofunctionality into hydrogels offers profound potential for the local regulation of valvular cell behavior in TEHVs and for *in vitro* tissue surrogates for investigating valvular biology in 3D. Toward this end, VICs have been grown atop and within hydrogels constructed from PEGDA,<sup>64,94</sup> methacrylated hyaluronan,<sup>63,91</sup> and gelatin.<sup>9</sup> These VICs adhere well to the hydrogels, proliferate, and produce ECM. They show substrate-dependent phenotype and differentiation,<sup>11,56</sup> and can interact with biofunctional groups integrated into the polymer network.<sup>10</sup> It should be noted, however, that incorporation of biofunctional groups can alter the material behavior of the hydrogel.<sup>40</sup> Because heart valves are layered structures in which the two outer layers are stiffer than the inner spongiosa layer, Tseng *et al.* recently reported a method for fabricating a quasilaminate tri-layer hydrogel in which the three layers can have distinct material behavior, biofunctionality, and cell density.<sup>99</sup> Another means of mimicking the mechanical behavior of heart valves is through combining hydrogels with electrospun mesh scaffolds, resulting in anisotropy and added stiffness that was shown to influence the adhesion, spreading, and cytoskeletal orientation of VICs.<sup>100</sup>

In conclusion, translating the *in vitro* results relating phenotype and microenvironment to the outcomes *in vivo* remains a challenge, since, the stiffness of the native leaflet, for example, far exceeds 25 kPa yet VICs do not exhibit a myofibroblast phenotype under homeostatic conditions. Another major challenge is understanding, yet alone controlling, the long-term remodeling of the valve matrix, whether acellular or cellular, after implantation.

## POLYMERIC HEART VALVES

Since the first polymeric flexible-leaflet heart valves were implanted in the 1960s, synthetic polymer-based

valves have been intended to combine the durability of mechanical valves with the hemocompatibility of bioprosthetic valves.<sup>17</sup> The initial results of implanting these valves led to a significant failure mainly due to their limited durability.<sup>82,84</sup> However, over the past decade, there has been significant progress toward development of these valves with improved hemodynamics and durability, and in some cases lower thrombogenicity. A “durable” polymer-based heart valve even with “mild” anticoagulation therapy may have the potential to offset the use of both mechanical (which require aggressive anticoagulation therapy) and bioprosthetic (which are not as durable) valves. Additionally, polymeric valves require a much lower production cost that make these valves more affordable compared to the other types of heart valves.

Here we use a broader definition for a polymeric heart valve as any valve whose leaflets’ material is made of polymer regardless of the housing/stent of the valve. The early polymeric valves were made of polyurethane. Since then, there have been various polymers used in a variety of valve types. These include valves made from Polysiloxanes, Polytetrafluoroethylene (PTFE) family, polyurethane, and polyvinyl alcohol (PVA).

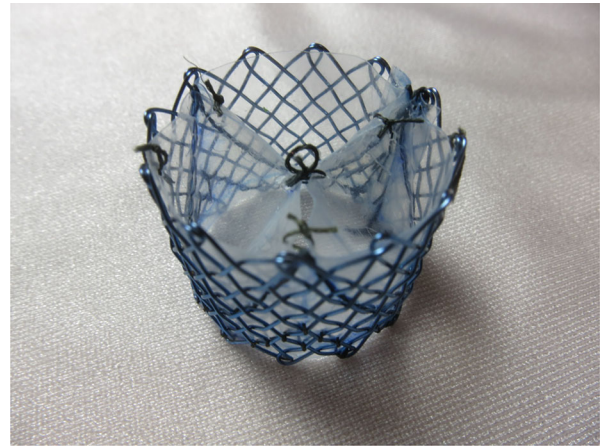
Polysiloxanes are silicone and oxygen based polymers, which are biostable with good elastic and flexural properties.<sup>30</sup> These materials were originally utilized for heart valves in the 1950s.<sup>83,84</sup> While these valves, with approximately 380  $\mu\text{m}$  thick leaflets, showed good *in vitro* performance and durability, they were highly thrombogenic, which resulted in serious post-surgical complications.<sup>80–82</sup> Durability has also been an issue with inconsistent fatigue properties between the same material but different batches.<sup>68</sup> Polytetrafluoroethylene (PTFE or Teflon) and its expanded version ePTFE are hydrophobic with smooth surfaces made from fluorinated polymers. Early experience with PTFE valves in the 1960s resulted in stiffening of leaflets and calcific nodule deposition.<sup>19,70</sup> Further investigations on ePTFE valves in the early 1990s also resulted in similar stiffening problems with macroscopic calcification.<sup>71</sup> Nevertheless, leaflets made from Gore-Tex<sup>®</sup> artificial pericardial (ePTFE) patches have been successfully used in conjunction with artificial conduits (Dacron based) for pediatric pulmonary surgical reconstruction during the Ross or other congenital heart defect repairs.<sup>5</sup>

Polyurethanes make up the most popular form of polymeric heart valves due to the ease of production. Over the years, these materials have gone through several iterations containing polyester, polyether, polycarbonate, and polysiloxane soft segments.<sup>31,55</sup> Early implants were plagued with stenosis secondary to fibrin deposition and thromboembolism.<sup>1</sup> Calcification

was a primary problem with polyurethane urea based valves implanted in juvenile sheep and calf studies.<sup>49,106</sup> Later development included valves made from polyether/PDMS based polyurethane<sup>31</sup> aiming for reduced leaflet stress and improved durability.<sup>44</sup> Jansen *et al.*<sup>53</sup> developed the J-3 polyurethane (an aliphatic PCU) valve with high effective orifice areas that tested for more than 600 million cycles in accelerated wear testing. However, calf studies were plagued with thrombus and calcification.<sup>14,53,54</sup> Other polyurethane valves based on polycarbonate urethane could go over 1 billion cycles in accelerated testing *in vitro*.<sup>34,36,85</sup> Once implanted in calf, severe calcification was observed that led to congestive heart failure and thrombosis in the animals.<sup>35,36</sup>

The next generation of polyurethane valves came with valve leaflets made from Estane (a PEU) and Lycra (a PEUU).<sup>12,13,15,16,61,104,105</sup> These valves showed accelerated calcification and their leaflets tore due to the presence of low molecular mass extractables.<sup>12,13,105</sup> A more recent iteration of these valves evolved into a conico-spherical design with symmetric leaflet opening and closing and better performance characteristics<sup>22</sup> and material, i.e., with different grades of the PEU and PEUU. These valves had Modulus of about 10 MPa with 73–111  $\mu\text{m}$  thick leaflets and lasted more than 300 million cycles. Following these improvements, *in vivo* performance in adult sheep demonstrated no evidence of thrombus formation, fibrin deposition, calcification or thromboembolism.<sup>16,104</sup> Nevertheless, extended exposure to water promotes degradation of soft segment polyurethanes due to potential hydrolysis of carbamate groups.<sup>24</sup> Polyurethane valves are manufactured primarily by either dip-casting or thermoforming, with very little hemodynamic difference as a result of the two methods,<sup>59</sup> but the dip-casting outperforms thermoforming in durability characteristics.<sup>29</sup> A new development is the polyhedral oligomeric silsesquioxanes-polycarbonate soft segment nanocomposite based heart valve with reduced platelet adhesion characteristics.<sup>43,55</sup> These valves varied in leaflet thickness in the range 100–200  $\mu\text{m}$  and had tensile strength in the range 31–55 MPa with Young's Modulus in the range of 15–26 MPa, and have shown lower transvalvular pressure drops ( $< 10$  mmHg), regurgitation ( $< 8$  mL/beat), and energy losses than bioprosthetic valves (EOA 2–3  $\text{cm}^2$ ).<sup>77</sup>

More recently, a new framework to manufacture hemocompatible polymeric leaflets for heart valve applications has been introduced that is based on a material comprised of interpenetrating networks (IPNs) of Hyaluronan (HA) and Linear Low Density Polyethylene (LLDPE).<sup>74</sup> HA is a naturally occurring polysaccharide that has a large unbranched structure consisting of repeating disaccharides of *N*-acetylglucosamine and glucuronic acid that makes it highly



**FIGURE 4.** Polymeric valve prosthesis assembled as a transcatheter aortic valve configuration using LLDPE developed by Dasi Group at Colorado State University. The heart valve was constructed using double layer LLDPE sheet sutured as three leaflets within a cage made of braided Nitinol wires acting as collapsible stent.

hydrophilic and anionic. HA is present in tissues and body fluids of all vertebrate animals with relatively high concentrations in native heart valve leaflets, particularly at the regions of the valve that are subject to compression.<sup>47,52</sup> These polymeric valves involve swelling plain LLDPE sheets in a solution of Silylated-HA, which is then cross-linked to itself before it is reverted back *via* hydrolysis to the native HA (Fig. 4). The leaflets made of this treatment show no change in bending stiffness comparable to natural fresh leaflets. The bending stiffness of the LLDPE/HA IPN films was not significantly different from that of natural HV leaflets (in  $\text{nN m}^2$ ): fresh leaflet<sup>102</sup> was  $6.3 \pm 2.82$ , fixed leaflet<sup>102</sup> was  $13.87 \pm 8.06$ , and the LLDPE/HA IPN samples ranged from  $12.93 \pm 2.34$  to  $26.11 \pm 3.62$ . While trending slightly higher they are not statistically significantly different ( $p < 0.05$ ) from the fresh or fixed tissue. HA-LLDPE IPNs were more hydrophilic than LLDPE controls, which resulted in less blood clotting and reduced cell adhesion compared to the plain LLDPE control. The prototypes of HA/LLDPE IPNs polymeric valves demonstrated an acceptable regurgitation fraction of  $4.77 \pm 0.42\%$ , and an effective orifice area (EOA) in the range  $2.34 \pm 0.5 \text{ cm}^2$ . These results promise compelling potentials for IPNs between HA and polymers as a hemocompatible heart valve.<sup>74</sup> Nevertheless, *in vivo* experiments are required to assess durability and calcification potentials.

## CONCLUSIONS

The field of heart valve tissue engineering is an emerging area of research and innovative solutions are



underway, and aim to fulfil the dream of developing self-regenerating heart valves. The realization of polymeric heart valves still remains a challenge given the long track record of initial promises followed by failures at the pre-clinical stages. While we are entering the promising and exciting area of biomolecule enhanced polymers such as with hyaluronan, it would be interesting to watch how such polymeric valves perform in pre-clinical trials specifically related to mineralization, need for anticoagulation and durability. Only time will tell the true potential for these non-traditional technologies, however, they have great potential to revolutionize the treatment of heart valve disease.

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