Assessment of a Siloxane Poly(urethane-urea) Elastomer Designed for Implantable Heart Valve Leaflets

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Synthetic polymer leaflets in prosthetic cardiac valves hold the potential to reduce calcification and thrombus, while improving blood flow, durability, and device economics. A recently developed siloxane poly(urethane-urea) (LifePolymer™, LP) exhibits properties essential for heart valve leaflets, including low dynamic modulus, high tensile strength, minimal creep, and excellent biostability. LP properties result from carefully designed "linked co-macrodiol" chemistry that maximizes silicone content and virtual crosslinks between soft and hard phases. Characterization of multiple commercial batches demonstrates

a robust synthesis process with minimal variation. Extensive ISO 10993-based biocompatibility testing resulted in no observable toxicity or other adverse reactions. An *ex vivo* AV shunt thrombogenicity investigation revealed nearly undetectable levels of platelet attachment and thrombus formation on LP surfaces. Chronic ovine implantation of prototype heart valves with LP leaflets showed no differences in thrombogenicity or systemic tissue response when compared to a clinically standard tissue-based valve. Toxicological risk assessment, based on extractables and leachables analysis of LP-based heart valves, confirmed minimal toxicological risk. Lastly, 24-week, strain-accelerated *in vivo* LP biostability testing confirmed previous favorable *in vitro* biostability findings. These studies demonstrate that this newly developed elastomer exhibits ideal biomaterial properties for the flexible leaflets of a totally synthetic heart valve replacement.

1. Introduction

As $(AB)_n$-type segmented block copolymers, polyurethanes (PUs) are comprised of three primary building block chemistries in tailored covalently linked sequences to yield polymer chain segments. These PU building block chemistries generally are: 1) macrodiol or polyol, 2) diisocyanate, and 3) chain extender. Myriad chemical combinations of these three components, each with several different chemistry possibilities, result in a substantial array of PUs with interesting ranges of useful properties.[1,2] Tailored PU mechanical and chemical properties derive from their molecular-level construction that yield distinct hard segments and soft segments which self-organize based on intermolecular forces (e.g., chain hydrogen bonding), segment association, and relative immiscibility to create microphase-separated polymer solids. PU microphase separation unique to each selected segment chemistry has long been proposed as essential to their compelling mechanical (e.g., tensile strength, elastic properties, hardness) and chemical behaviors (e.g., gas/water permeability, hydrolytic stability, biocompatibility).[1-3]

Biomedical PUs enjoy a substantial history of materials development and track records of success across a diverse palette of medical products.[4] Numerous academic and clinical medical implant development experiences have demonstrated how PU chemistry is related to both its biocompatibility and biostability. Adoption and re-purposing of early PU materials from other commercial applications for medical device use resulted in many successes and a small number of notable performance challenges. Specifically, commercialized polyetherurethane-based pacemaker lead insulation coatings and breast implant porous coatings both exhibited device-associated failures attributed to polymer degradation mechanisms catalyzed under physiological conditions.[5] Oxidative degradation of the polyether soft segment associated with PU elastomeric properties was considered deleterious to these early implanted PUs.[6,7] This link between PU segment chemistry and resulting *in vivo* PU implant performance served to distinguish the unique *in vivo* environmental demands for biomedical PUs, and motivated extensive and continuing efforts to improve PU biostability for long-term implantation.[4]

One approach to reducing polyether-based PU oxidative degradation susceptibility has exploited new polyether polyol building blocks with fewer ether groups, such as poly(hexamethylene oxide) (PHMO), which has been shown to improve *in vivo* stability and, importantly, serve also to improve chemical compatibility of apolar siloxane (e.g., polydimethylsiloxane oligomers, PDMS) soft segment building blocks.[4, 8-12] Substantial recent developments, including intelligent selection of PDMS terminal functional groups and the use of a second macrodiol as a segment compatibilizer, have enabled controlled phase mixing between PU soft and hard segments with superior mechanical properties and enhanced PDMS-PU *in vitro* hydrolytic resistance and *in vivo* biostability.[4, 13-16] Latest generation commercially available PDMS-based biomedical PUs utilize a two-step bulk polymerization process, allowing scalable mass production without metal catalysts and improved mechanical characteristics and biostability suitable for medical devices (e.g., Elast-Eon 2A®, or E2A,

AorTech Biomaterials, UK).[17,18] E2A‟s superior biostability versus conventional polyether PU Pellethane® 2363-80A has been shown in numerous ovine subcutaneous implantation studies.[16, 19-22] A translational success story, Abbott‟s E2A-based Optim® cardiac lead insulation has been clinically implanted since 2006, with over 6 million patients benefiting worldwide.[23]

Despite their clinical success, recent *in vitro* studies using Arrhenius-accelerated hydrolysis have raised concerns over long-term siloxane-based PU biostability.[24-27] A recognized primary limitation of Arrhenius-type, temperature accelerated, *in vitro* degradation models is the high and sustained testing temperatures producing PU microstructural and morphological changes not observed *in vivo*.[28] Most significantly, temperature-accelerated *in vitro* PU degradation results do not correlate with the very minimal changes seen in 5-year and 8 year clinical explants of Optim® (E2A) pacemaker leads.[29,30] An actively monitored registry study of the first commercially released Optim cardiac leads, encompassing 14,500 devices over 73,000 patient years, confirms Optim‟s *in vivo* stability with an outstanding all-cause device survival rate of 98.3% after more than 12 years of implantation.[23] Given significant ambiguities in accelerated *in vitro* testing to predict actual PU *in vivo* stability, future focus should be on improving these *in vitro* models and validation against long-term implant studies. Establishing strong *in vitro-in vivo* correlations is required to confidently accelerate further development of siloxane-based PUs central to several important medical device classes in clinical use, including prosthetic heart valves.

Prosthetic heart valves are well-established, lifesaving medical devices. While complications such as thrombosis and calcification do occur, the rates are relatively low and valve performance and longevity is considered acceptable. The majority of heart valves implanted today feature leaflets made with fixed exogenous cardiac tissue, i.e. bovine pericardium or porcine valve leaflet. A smaller fraction of implanted heart valves utilize a rigid mechanical design featuring one or more pivoting carbon discs/leaflets. Carbon valves

are acknowledged to be extremely durable, however, due to the non-physiological flow and material surface chemistry, strong anticoagulation therapy is required and hemolysis is a concern. Tissue valves require only mild anticoagulation therapy, demonstrate good longevity, and can be delivered by a minimally invasive catheter, however calcification complications remain a concern.

Because polymer properties can be carefully tuned and controlled, the possibility of using polymeric leaflets in prosthetic heart valves has been carefully explored by multiple organizations. In addition to physical properties, the geometry of polymer valve leaflets can be intentionally designed to ensure ideal physiological function, approaching that of native leaflets. Therefore, molded or solution processed polymer heart leaflets offer the potential for low cost, highly-controlled, physiologic, minimally invasive, prosthetic valves with excellent hemodynamics. Historically, many polymers have been explored as leaflet materials, including silicone rubber, polytetrafluoroethylene (PTFE), expanded PTFE (ePTFE), polyvinylalcohol (PVA), poly(styrene-isobutylene-styrene) (SIBS), and an array of polyurethanes.[31] However, to date, no prosthetic valve with polymeric leaflets has achieved commercial success. Familiar clinical issues such as hemodynamics, thrombosis, and calcification, as well as *in vivo* degradation have been the primary challenges.

New PDMS-PU chemistries continue to be reported, seeking to improve *in vivo* biomaterials performance using PU structure-property relationships.[4] Arguably, siloxane-containing PUs exhibit the best biostability and biocompatibility to date, but frequently lack state of the art mechanical properties. Application-specific properties for medical devices require control of specific PU mechanical properties together with robust, reliable biostability under implant conditions. Synthetic heart valve leaflets, for example, require high tensile and tear strengths, high resistance to creep deformation, and specific dynamic moduli. PUs were not capable of delivering these requisite properties until siloxane-based PUs with controlled

microphase morphologies using new molecular and segmental architectures were produced, surpassing known mechanical properties for E2A.[32-35]

This study reports chemical, *in vitro*, and *in vivo* analysis of a first-in-class biomedical grade siloxane-based poly(urethane-urea) (SiPUU) (LifePolymer®, LP, Foldax, USA) specifically developed for synthetic leaflets in a prosthetic heart valve (Tria™, Foldax, USA). After significant development efforts, which were thoroughly described in a series of publications, LP met property goals established to ensure proper and safe mechanical function of Tria valve leaflets, including ≥30 MPa UTS, ≥500% elongation, ≥50 N/mm tear strength, ≥200 J m^{-2} tear energy threshold, 25-35 MPa dynamic modulus, creep rate < E2A, and biostability ≥ E2A.[32,35,36] LP‟s published synthesis and chemical design exploits 5 distinct pre-polymer building blocks, including a diamine chain extender and siloxane-containing macrodiol and hard segment components, facilitating co-macrodiol soft segment interactions with hard segments using both carbamate and urea chemistry.[32-35] LP soft segments are based on a 20:80 (w/w) mixture of 4,4‟-methylenediphenyl diisocyanate (MDI) linked PHMO and telechelic α,ω-bis(6-hydroxyethoxypropyl)-PDMS. LP hard segments represent nearly 45% (w/w) of total mass, comprising MDI and a mixture of 1,2-ethylenediamine (EDA) and 1,3-bis (4-hydroxybutyl)-1,1,3,3-tetramethyldisiloxane (BHTD).[32] The creative urethane-urea polymer construction distinguishes this siloxane PU from E2A and another analogous commercial biomedical siloxane-based PU, PurSil35 (DSM Biomedical, NL), that each contain 4 linking components, but, notably, lack BHTD and the urea linking chemistry that results from the inclusion of EDA. Innovative multi-step, scalable polymer processing yields ~50% PU siloxane content, a silicone-rich PU surface, and high levels of hydrogen-bonded inter-segment interactions between soft and hard segment phases.[32,34] LP‟s molecular design is responsible for the demonstrated superior mechanical features and biostability necessary for use as a synthetic prosthetic heart valves leaflet.[32-35] Building on the previously published exploration and characterization data, this study provides a wide range of additional *in vitro*

and *in vivo* evidence supporting LP in this promising cardiovascular device application. These data include further material characterization, batch-to-batch consistency, ISO10993 biocompatibility, *ex vivo* thrombogenicity, extractables and leachables toxicological assessment, chronic ovine valve implants, and accelerated *in vivo* biostability.

2. Results

2.1. Characterization of production-scale SiPUU

An initial 14 LP synthetic batches have been completed commercially. Each was thoroughly characterized to ensure a consistent, high quality polymer with specifications necessary for valve leaflet production. Data from these batches are presented in **Table 1** and examples of the raw data are included in the Supporting Information. While many specifications remain proprietary, the inter-batch production data demonstrate robust consistency. Standard deviations of the polymer mechanical properties are all within 6% of the averages and T_g standard deviations are <3 $^{\circ}$C. Even polymer molecular weight GPC data, typically acknowledged to produce method-based variability +/-10%, is within 16% of the mean value. Notably, molecular weight, ultimate tensile strength, and elongation averages are significantly above those reported for earlier SiPUU lab-scale development, while PDI remains consistent, indicating an improvement in process capability at the larger scale.[32] Dynamic modulus, a critical parameter to ensure proper leaflet movement, blood flow, and overall valve sealing, is remarkably consistent and well within the 25-35 MPa target range identified during material development and valve leaflet specifications. Tear energy threshold, defined as the energy level that can be cyclically applied to a leaflet without propagation of a cut/tear, easily exceeds the minimum design requirement of 200 J m^{-2} and provides a >2x margin of safety during aortic valve function.[37]

2.2. Aortic Valve Fabrication

The Foldax Tria™ Aortic Valve design (**Figure 1**) is a 100% synthetic polymer prosthetic aortic heart valve repeatedly fabricated for pre-clinical and biocompatibility testing. The valves consist of LP leaflets solution-cast onto a radio-visible PEEK stent and a PTFE felt sewing ring secured with polyester suture. Sterilization was achieved by standard ethylene oxide methods. The Tria valves fabricated for this study underwent all manufacturing processes/controls and met all visual, mechanical, and dimensional specifications necessary for human use.

2.3. Biocompatibility

Because a complete medical device best represents patient exposure, biocompatibility testing described in this study was performed on a complete, sterilized Tria heart valve with an approximate composition of 40wt% LP, 40wt% PEEK, and 20wt% PTFE. Biocompatibility assessments were completed in compliance with ISO 10993 standards and GLP regulations. A summary of each test result is provided in **Table 2**. All biocompatibility testing met the relevant criteria, identifying no significant risk of toxicity or tissue injury. Successful ISO 10993 biocompatibility results, such as those detailed for the Tria valve with LP leaflets, are necessary to ensure patient safety and secure regulatory approval of implantable medical devices worldwide.

2.4. Toxicological risk assessment from exhaustive extraction and leachables analysis of Tria valves

GC-MS and LC-MS analysis of exhaustive Tria valve extracts (polar, semi-polar, apolar) identified low levels of 37 unique compounds and 24 groups of related compounds (**Table 3**). Identical analysis of PBS and ethanol/water simulated-use leachables solutions identified low levels of 33 unique compounds and 11 groups of related compounds (Table 3). Examples of

compound groups included ethylene glycols, fatty alcohols, siloxane cyclics, siloxane oligomers, DMAc-related compounds, MDI-related oligomers, and MDI-diol-siloxane related oligomers. The identification of very low levels of extractable compounds is typical, even for implant-grade polymers, due to the high sensitivity of these analytical methods.

As an outcome of the comprehensive toxicological assessment, Margin of Safety (MOS) values were calculated for each of the unique compounds and related compound groups identified by GC-MS and LC-MS. MOS values ≥1 indicate a minimal and acceptable risk of toxicity when the device is used as intended. MOS values <1 require additional consideration to make a final toxicity determination. Minimal toxicity (MOS≥1) was confirmed for all compounds leachable under simulated-use conditions and those compounds identified with polar and semi-polar solvents under aggressive, exhaustive extraction conditions. Only under the non-physiological conditions of an exhaustive hexane solvent extraction at 50 °C for 72 hours were compounds identified with a MOS less than 1.0, ranging between 0.5 and 0.9.

ICP-MS identified eight metals, Mg, Al, B, Na, Si, K, Ca, and Ba, found in quantities of at least 1µg/device in exhaustive water extractions, PBS leachables analysis, and ethanol/water leachables analysis. The MOS of these elements was remarkably high, ranging from 79 to 501,400. As a result, no toxicity concerns were related to extractable or leachable elements.

All available data were then considered to assess overall toxicology risk for the Tria valve with LP leaflets. Acknowledging the successful completion of ISO10993 biocompatibility testing (including acute systemic toxicity and genotoxicity), a minimal toxicity assessment from the simulated use leachables analysis, and the understanding that the MOS from exhaustive extractions significantly overestimates potential biological exposure, the Tria valve with its three LP leaflets was concluded to represent minimal toxicity risk.

2.5. Subchronic preclinical histological evaluation of implant Tria valves with LP leaflets in sheep

Eight (8) sterilized Tria valves (23mm) with LP leaflets were successfully implanted in the aorta of 8 sheep for a 140 day period. Human Carpentier-Edwards Perimount™ aortic valves (model 2900-25mm) were implanted in 2 sheep as controls.

No significant health problems were observed for the 10 valve-implanted sheep during the course of the 140 day study. Clinical chemistry data identified no significant trends. All animals gained weight throughout the study. No mineralization, hemorrhage, or valve surface thrombus was observed on either LP or control valves. Additionally both valve types demonstrated only minimal inflammation adjacent to the sutures and the aorta were found to be patent with no thrombi. The LP leaflets supported no fibrous tissue (pannus) or endothelialization. The control leaflets demonstrated a mild amount of fibrous tissue (pannus) coverage, but no endothelialization was observed. Overall, the assessed gross and microscopic histopathological parameters (**Figure 2**) of the Tria valve and adjacent tissues were similar or superior for the LP test articles compared to the control articles, had minimal severity, and did not create any clinically significant findings or safety concerns in this animal model.

Histopathological assessment of organs and tissue not in close proximity to the valves (kidneys, lungs, liver, gallbladder, spleen, lymph nodes, and brain) did not identify any notable findings unique to the Tria valve.

2.6. *Ex vivo* non-human primate AV shunt thrombogenicity

The *ex vivo*, non-human primate (NHP) AV shunt method previously described by Harker and Hanson was successfully employed to evaluate thrombogenicity of LP grafts (n=6) and ePTFE control grafts (n=5) **(Figure 3)**.[38] Overall platelet deposition on LP grafts was minimal (<0.08x10^9 platelets) for the entire 60 minute study period (**Figure 4**). This low platelet deposition is an order of magnitude below historical published results on ePTFE grafts with clopidogrel (20 mg kg^{-1}) and aspirin (10 mg kg^{-1}) which had 2.0±0.4x10^9 platelets after 60 minutes.[39] A statistical comparison of LP and ePTFE graft platelet deposition confirmed that ePTFE grafts underwent significantly ($p < 0.05$ in t-test) higher platelet attachment at 30, 45, and 60 minutes.

Fibrin accumulation on 4 of 6 LP grafts was sufficiently low to be below the gamma counter detection limit. The average amount of fibrin present on the LP devices was 0.003±0.007 mg, orders of magnitude lower than the average ePTFE device fibrin deposition of 0.844±1.156 mg. A single ePTFE replicate exhibited >8x the fibrin accumulation (2.897 mg) of the average of the other four (0.331 mg). No justification for this outlying data point could be identified. The resulting standard deviation of the ePTFE fibrin data with this outlier was high and statistically significant differences vs LP were not identified (t-test, $p = 0.179$). Removal of this outlier from the ePTFE dataset results in a statistically significant difference ($p = 0.027$) between the LP and ePTFE samples.

2.7. *In vivo* biostability from pre-strained LP films in rabbit subcutaneous implants

Pre-strained LP and control polyurethane (Pellethane 80A and Pellethane 55D) dumbbells were successfully implanted subcutaneously above the dorsal muscle of eight (8) rabbits for 3-6 months. Sixteen (16) of each sample type were implanted. Of the 48 implanted samples, surgical complications or infection required 10 samples (3 LP and 7 controls) to be explanted and removed from the study within 30 days of implant. No other implant-related health issues were noted during the study.

Figure 5 details the results of visual examination scoring, which attempts to classify the extent of any visible degradation. The 6 month rabbit model was successful in causing significant degradation (score of 6) in the positive control samples. A moderate amount of surface degradation was even identified in the negative controls (score of 4). Note that the Pellethane 55D Negative Control is a biomaterial successfully implanted clinically for decades, but has been shown to demonstrate mild degradation *in vivo* and in accelerated *in vitro* testing.[30,40] Our control results confirm that the 150% strain level in this rabbit model is a strong accelerator of PU degradation. Even with this level of acceleration, the LP samples showed no visual signs of degradation (score of 1).

Figure 6 provides typical FE-SEM images and general observations for the LP and control groups after 6 months. Note that due to the challenges associated with assessing transparent sample surfaces with light microscopy, FE-SEM assessments may deviate somewhat from visual evaluation results of the same samples. The positive controls (Pellethane 80A) show significant surface pits, cracking, and complete separation of the central region of one sample. The negative controls (Pellethane 55D) showed minor surface pitting and cracking at both time points. Note that the FE-SEM imaging identified less pitting and shallow cracking in the negative control than the visual examination. The LP test articles showed no signs of pitting, cracking, or any other types of degradation even at 6 months.

These study data show that the LP material is highly biostable, even in an *in vivo* model accelerated by strains that are >10X the expected strain in a valve leaflet. Additionally, FE-SEM-EDAX analysis found no evidence of calcium deposition on the LP test article at either 3 or 6 month time points (data not shown).

3. Discussion

A completely synthetic and clinically reliable heart valve prosthesis has been elusive, requiring highly complex combinations of valve design, biomaterials combinations, manufacturing constraints, implant tools, and surgical techniques. The newly designed Tria aortic replacement valve contains elastomeric polymer leaflets of a new synthetic siloxane poly(urethane urea) (SiPUU) commercialized as LifePolymer™ (LP, Foldax, USA). A series of previous publications has extensively described LP‟s synthetic chemistry, cast bulk film microstructure, silicon surface enrichment, unique polymer processing, and extensive physical properties relevant to synthetic heart valve application.[32-35] Replacement of traditional xenogeneic pericardial tissue leaflets with high performance SiPUU provides the advantages of a highly controlled leaflet design, ideal and tailored mechanical properties, a robust supply chain, and lower manufacturing costs. Nonetheless, translation, clinical testing and commercialization of any replacement heart valve requires development and testing according to a number of guidance and standards documents, including ISO 10993 “Biological Evaluation of Medical Devices”, ISO 5840-1 “Cardiac valve prostheses: general requirements”, and ISO 5840-2 “Cardiac valve prostheses: surgically implanted heart valve substitutes”. Results from a series of studies intended to validate the biocompatibility, safety, and stability of the LP leaflet material and the Tria aortic valve prosthesis are aligned with these guidances. Together with the earlier reported work on this new SiPUU copolymer, an exhaustive data set now confirms LP‟s suitability for use as a leaflet in a replacement heart valve. [32-35]

Many important chemical, thermal, and physical properties of LP made at the laboratory scale have been described during the LP biomaterial"s early development. [32-35] These include important benchmarks for LP"s solvent processable mechanical properties (elasticity, tensile strength, tear strength, creep) attributed to unique co-macrodiol chain-extended segmental interactions with new hard segments also containing siloxane chemistry, cast surfaces consistently and substantially enriched in siloxane low energy blocks, and both carbamate and urea linkages imparting stability and regular hydrogen bonding.[33,34] Extending this bench synthesis to the first stages of device translation, multiple LP batches commercially synthesized at scale demonstrate a high level of process control. Modest enhancements in molecular weight, ultimate tensile strength, and elongation over previously reported lab-scale properties further indicate improvements in process capability at the larger scale.

The ISO 10993 biological evaluation standards are adopted globally as part of a risk management process for verifying the biocompatibility of finished medical devices, including heart valves. Because a heart valve remains in long term blood and tissue contact, ISO 10993-1 requires the most rigorous battery of tests, including cytotoxicity, intracutaneous reactivity, material mediated pyrogenicity, systemic toxicity, implantation effects, hemocompatibility, and genotoxicity. A carcinogenicity assessment is also expected if the device contains any known mutagens, carcinogens, or reproductive toxins.

The Tria valve with LP leaflets passed all standard biocompatibility testing, identifying LP in this context as non-cytotoxic, a non-irritant, a non-sensitizer, non-pyrogenic, non-genotoxic, non-clastogenic, and producing no acute systemic toxicity (Table 2). Longer term subchronic toxicity testing in an ovine model identified no risk of localized or systemic toxicity. The Tria valve materials were also determined to be non-genotoxic by both standard AMES and Mouse Lymphoma methods. These results and the absence of any known genotoxic or carcinogenic compounds in the LP formulation, justified not performing a carcinogenicity test, in agreement with ISO 10993-1 guidelines.

As per ISO 10993-4, the blood compatibility of any heart valve must be assessed with both hemolysis and *in vivo*/*ex vivo* thrombosis studies. As shown in Table 2, the Tria valve materials were non-hemolyzing and demonstrating acceptably low rates of coagulation with PTT and complement activation *in vitro* studies. The *ex vivo* thrombosis assay using the LP-based A-V NHP shunt (Figure 4) further supported low coagulation activation by LP, producing nearly undetectable levels of platelet attachment and fibrin deposition *in situ*, both key markers of thrombosis. Essential for any heart valve leaflet material, the Tria valve and LP leaflets have both shown exceptional hemocompatibility in two different large animal models (see Figures 2 and 4). This is attributed to high, non-stoichiometric levels of siloxane at the LP leaflet surface, known to favor hemocompatibility.[32]

A substantial fraction of adverse effects from implanted medical devices are attributed to the unintended release of compounds or elements.[40] Polymeric biomaterials in medical devices may contain extractable and potentially toxic low molecular weight species, such as oligomers, plasticizers, antioxidants, and processing aids. For this reason, ISO 10993 biocompatibility testing uses extraction techniques to isolate these compounds for biological assessments. A typical complementary analytical approach described by ISO 10993-18 is the identification, quantification, and toxicological assessment of compounds and elements that are extractable under simulated-use conditions (leachables) and/or using aggressive extraction solvents and conditions, an "exhaustive extraction". In this manner, the toxicological safety of a device can be confirmed not only with biological testing but by state-of-the-art chemical analysis, such as GC-MS, LC-MS, and ICP-MS, coupled with toxicological risk assessment.

It is important to recognize that the calculation of the MOS value is only one element of a toxicological assessment and it involves multiple highly conservative assumptions. First, the calculation assumes that 100% of each compound extracted would be bioavailable within 24 hours, with the same quantity released daily over the lifetime of the patient. Under actual use conditions, compounds from a one-time implant are expected to leach out over weeks, months,

or even years, at ever-decreasing concentrations, resulting in much lower toxicity risks. Additionally, under actual use conditions, many compounds identified specifically by this harsh apolar solvent extraction may never be released from the valve *in vivo*. This can be seen when comparing the exhaustive extraction data with the leachables data. Thus, the MOS calculations significantly overestimate potential biological exposure and toxicological risk.

After considering the entirety of the data, including the non-toxic assessment of leachable compounds and element, the toxicological data from the exhaustive extractions, and the successful ISO 10993-1 biocompatibility test results (Table 2), including acute systemic toxicity, genotoxicity, and *ex vivo* thrombogenicity (Figure 4), the Tria valve with its three LP leaflets was concluded to represent minimal toxicity risk.

Implant biocompatibility and biological safety, particularly in the context of a device mechanical function, as for heart valve leaflets, generally includes further assessment of material biostability. ISO 10993 parts -13, -14, and -15 describe testing protocols designed to identify compounds produced as a result of biomaterials degradation. *In vitro* studies are appropriate, if guidance documents or literature reports provide methodology relevant to the device and specific materials being considered. Previous publications demonstrate generally outstanding *in vitro* and *in vivo* resistance of LP to aggressive oxidative attack.[34-35] Many publications over the past 40 years use an accelerated *in vivo* model of polymer degradation for polyurethane elastomers.[20,42-43] The 6-month rabbit implant study described accelerates polymer degradation *in vivo* by applying a constant 150% strain to the material just prior to subcutaneous implantation. This level of strain is >10X the strain expected to be seen in an aortic valve leaflet. Consistent with previous studies this level of applied strain elicits significant pitting, cracking, and material degradation in a soft polyetherurethane, such as Pellethane 80A (Figure 6). Even Pellethane 55D, with successful clinical use in a variety of implant applications does not avoid degradation in this aggressive test (Figure 6). By contrast, the strained LP samples showed no signs of degradation (e.g., pitting or cracking) even after 6

months of implantation under these accelerated conditions. A similar level of biostability has been previously demonstrated by other siloxane-urethanes including ElastEon2A and PurSil, both of which have successful implant applications.[20,42] Addition of unique new urea segment bonds and a siloxane-containing BHTD chain extender to enhance LP‟s mechanical properties required for effective valve leaflet function has not compromised its *in vivo* biostability in this accelerated assessment.

4. Conclusions

Mechanical and biological performance requirements for heart valve prosthesis leaflets are very demanding. Despite the indisputable advantages of a synthetic polymer leaflet, no examples capable of reliable device commercial translation are known. Substantial prior work highlights focused development of a siloxane polyurethane-urea biomaterial intended solely for use as a heart valve leaflet.[32-35] Incorporating a high level of siloxane and optimized levels of urea linker chemistry, a linked co-macrodiol, and a silicone-containing hard segment, LP demonstrates an ideal dynamic modulus for leaflet use with a high mechanical strength, low creep, high tear energy threshold, and both *in vitro* and *in vivo* biostability. Scaled batch-to-batch polymer production consistency, excellent ISO 10993 biocompatibility and *ex vivo* thrombosis results, non-concerning toxicological risk assessments, successful chronic sheep implantation, and promising rabbit biostability testing results all confirm the biological safety of the LP biomaterial in the Tria valve device design. These new and promising results, together with the attractive original LP property set from lab-scale production, provide a diverse and substantial evidence base to proceed with the application of LP leaflets in a totally synthetic heart valve.

5. Experimental Methods

Siloxane-based poly(urethane-urea) (SiPUU) synthesis: As previously described,LP SiPUU was synthesized with a multi-step synthetic process.[32] Briefly, α,ω-bis(6-hydroxyethoxypropyl)poly(dimethylsiloxane) (PDMS) was purified in a wipe film evaporator to remove low molecular weight cyclic siloxane compounds. Linked PHMO was synthesized by reacting a 2:1 molar ratio of poly(hexamethylene oxide) (PHMO) and methylene diisocyanate (MDI) at 80 °C for 2 hours. Pre-polymer was prepared by blending together PDMS and linked PHMO, adding MDI titrated to ensure complete reaction of the polymer, and stirring for 2 hours at 80 °C. Chain extension began with the addition of 1,3-bis(4-hydroxybutyl)-1,1,3,3-tetramethyldisiloxane (BHTD) and a 2-hour reaction period. The final steps of chain extension were the dissolution of 10% w/v dimethylacetamide (DMAc) followed by the addition of ethylene diamine (EDA). Chain extension was complete over 3 hours. The resulting approximate molar composition of LP is 50% MDI, 3% linked PHMO, 17% PDMS, 15% BHTD, and 15% EDA. No processing aids, antioxidants, or other additives are present in LP. For testing purposes, LP films (200 μm thickness) were solution-cast from each batch of LP, using LP-DMAc solutions (25% w/w) applied to glass plates using a film casting knife, then dried at 55 °C overnight.

Molecular weight (M_w, M_n, *PDI*) of each batch of LP-DMAc solution was determined with a GPC system consisting of an HPLC pump/controller, RI detector, Styragel HT2, HT3, HT4, HT5 columns (Waters) at 80 °C, and a 1.0 ml min^{-1} flow of mobile phase (DMAc with 50 mM LiBr). Molecular weight values were reported relative to monodisperse polystyrene standards.

Glass transition temperature (T_g) was determined with a DSC (Discovery, TA Instruments). Dry film samples (3-5 mg) from each batch were equilibrated at -90 °C, then taken through heating (10 °C min^{-1}), cooling (5 °C min^{-1}), and reheating (10 °C min^{-1}) cycles between -90 °C and 100 °C. T_g values during heating, cooling, and reheating were determined.

Films from each LP batch were spectroscopically analyzed using a diamond ATR-FTIR system (iS50, ThermoFisher) over a spectral range of 525-4000 cm^{-1}, averaging 32 scans at a resolution of 4 cm^{-1}. Each spectrum was compared to a historical reference spectrum using a correlation coefficient calculation (QCheck, Omnic software). A coefficient of 1.0 indicates a perfect match.

Seven (7) replicate ISO 37 type 2 dog bones were cut from a cast LP film from each batch. Using a tensile testing system (Mark-10), dog bones were pulled in tension to failure at 100 mm min^{-1} under ambient conditions. Ultimate tensile strength (UTS) and elongation to failure were calculated for each LP batch.

For each batch, six (6) replicate samples (10 mm x 42 mm) were punched from a LP film and a cut of controlled depth was made on one edge of each sample. Using a mechanical tester (Electroforce 3200, Bose Instruments), these samples were subjected to a set of 50,000 tensile fatigue cycles at 10 Hz and a maximum stress of 0.5 MPa in 37 $^{\circ}C$ water. Samples were then subjected to sets of 50,000 fatigue cycles at increasing stress levels in 37 $^{\circ}C$ water until the crack/tear growth exceeded 0.1 nm $cycle^{-1}$, at which point a tear energy threshold (J m^{-2}) was calculated based on the energy of loading and the sample dimensions.[37] Dynamic modulus (MPa) was calculated as the slope between the minimum and maximum points on the stress-strain curve during cyclic loading at the 0.5 MPa stress level (**Figure S5**).

Aortic valve fabrication: The Foldax Tria™ Aortic Valve design (Figure 1) is a 100% synthetic polymer prosthetic aortic heart valve with a flexible, radio-visible polyetheretherketone (Zeniva® PEEK, Solvay) stent and annular base with diameters between 17 mm and 27 mm. Three flexible LP leaflets with specifically designed thickness profiles were fabricated in place, on the frame, using a carefully controlled solvent casting process. Dipping speed and orientation, LP polymer solution (in DMAc) viscosity, environmental temperature and humidity, and drying parameters were critical to ensuring that cast leaflets

meet dimensional specifications with micron-level precision. After polymer film casting, a mechanical trimming step was performed to finalize LP leaflet dimensions. A PTFE felt sewing ring was secured onto the annular PEEK valve base using a polyester suture running through a series of regularly spaced holes in the base outer annulus. Sterilization was achieved by standard ethylene oxide methods.

Biocompatibility assessments: A series of ISO 10993-based biocompatibility tests was performed on sterilized Tria Aortic Valves with LP leaflets. A complete Tria heart valve consists of LP leaflets (~40% w/w), a rigid frame made of implant grade PEEK (~40% w/w), a sewing ring (~20% w/w) made of implant grade PTFE secured with a commercially available polyester suture. Table 2 provides an overview of the specific tests performed, all of which were performed in compliance with FDA Good Laboratory Practice (GLP) regulations (21 CFR Part 58). Summaries of the test methods are provided in **Table S2** in the Supporting Information.

All preclinical biostability studies were performed in accordance with the United States National Institutes of Health (NIH) Guide for the Care and Use of Laboratory Animals, which includes approval by the test facility Institutional Animal Care and Use Committee (IACUC). The test facility was registered with the United States Department of Agriculture (USDA, #41-R0074) and accredited by the Association for the Assessment and Accreditation of Laboratory Animal Care (AAALAC, aaalac.org).

Toxicological risk assessment from exhaustive extraction and leachables analysis: Potential toxicological risks of the Foldax Tria Aortic Valve, including the LP leaflets were assessed using exhaustive extraction performed in triplicate with water (polar), 50% ethanol/water (semi-polar), and hexane (non-polar) solvents, all at 50 $^{\circ}$C for 72 h. Any compounds/elements extracted from the valves were identified by QTOF-GCMS, QTOF-

LCMS, and ICP-MS. Relative quantification was performed in triplicate by QTOF-GCMS and UHPLC-CAD-UV. See the Supporting Information for additional analytical method details. Grouping of compounds was performed when consistent toxicological data were available. The total concentration per device for a compound group represented a sum of the amounts for each individual compound within a group. All compounds found with maximum levels above the analytical evaluation threshold (AET) of 1.5 μg device^{-1}, a limit supported by the ICH M7 guidance, were reported. A reporting limit of 1.0 μg device^{-1} was used for ICP-MS elemental data. Data from the exhaustive extractions were key inputs in the subsequent assessment of toxicological risk using methods consistent with ISO 10993-17:2002 R2008.

A biologically relevant, simulated-use leachables analysis was also performed in triplicate, using both PBS (polar) and 40% ethanol/water (semi-polar) as extraction solvents at 37 °C for 72 h. Identification and quantification of compounds was performed in an identical manner as the exhaustive extraction described above.

The toxicological evaluation/risk assessment process for both leachables and exhaustive extraction was performed by an experienced toxicologist and followed the general approach described in ISO 10993-17:2002 R2008. The identification and characterization of potential deleterious effects of exposure to extractable chemicals/elements included the establishment of a tolerable intake, TI [μg kg^{-1} day^{-1}], for the chemicals/elements and calculation of the human dose equivalent, HDE [μg kg^{-1}], and a margin of safety, MOS = TI/HDE. Due to the demonstrated non-mutagenic nature of the Tria heart valve, the default TI used for compounds without alternative data was 1.5 μg kg^{-1} day^{-1}, the Cramer class III Threshold of Toxicological Concern (TTC).

Subchronic toxicity (140 day) preclinical evaluation: Tria aortic valves with LP leaflets were evaluated in the healthy ovine model over an implant period of 140 days. Sterilized Foldax Tria valves (23 mm) were implanted in 8 animals (n=8 test samples). A sterile human

Carpentier-Edwards Perimount™ aortic bioprothesis model 2900-25 mm was implanted in 2 animals as a clinically acceptable, benchmark control device (n=2 controls).

Animals were screened for compatible annulus size prior to enrollment. A total of ten (2 female, 8 male) sheep, 40-60 kg in weight, underwent a left thoracotomy and were prepared for cardiopulmonary bypass. Once on bypass, the aorta was opened and the test or control device was implanted as per the manufacturer instructions. During the procedure, activated clotting time was maintained above 250 s by the administration of intravenous heparin, as needed. The aorta was then sutured closed and the animals were weaned off bypass. No post-op anticoagulant therapy was administered. General health and clinical chemistry (CBC, serum chemistry, LDH, haptoglobin, free hemoglobin, reticulocyte count, coagulation panel) were assessed regularly, beginning at day 7 and monthly thereafter. At approximately 140 days post-implant, the animals underwent a sedated terminal procedure and a gross necropsy was performed. The valve, heart and lungs, as well as tissues from the lymph nodes, liver, spleen, kidney, and brain were collected for histological evaluation. All histopathological data collected from the Tria aortic valve were compared directly to the control valve.

This preclinical study was performed in accordance with the NIH Guide for the Care and Use of Laboratory Animals, which includes approval by the test facility IACUC. The test facility was registered with the USDA (#41-R0074) and accredited by AAALAC (aaalac.org).

Ex Vivo AV shunt thrombogenicity study: Six (6) LP grafts (4 mm diameter, 70 mm long) were prepared by dip-coating a stainless steel mandrel in a 22% (w/w) LP/DMAc solution. Each sample was dip coated 4 times in a glove box at 85 %RH. Between each coating, samples were rotated in an oven at 55 $^{\circ}$C for 30 min. After the final coating, samples were held in a 55 $^{\circ}$C oven overnight. LP grafts were removed from the mandrels under running RO water. Grafts were packaged and sterilized by ethylene oxide.

Silicone rubber tubing (4.0 mm ID, 6.5 mm OD) was abutted to both ends of the grafts and bonded with silicone medical adhesive and an overlying 15 mm length of FEP heat shrink tubing (Figure 3).

The *ex vivo*, nonhuman primate (NHP) AV shunt method previously described by Harker and Hanson was employed to evaluate vascular graft thrombogenicity.[38] A single baboon with no history of anticoagulation or antiplatelet therapy was utilized for this study. Six LP grafts were tested over the course of two days. CBC values and platelet counts were confirmed to be normal prior to the start of each study. A silicone rubber tubing A-V femoral shunt was surgically established. Autologous ^{111}In-labeled platelets and ^{125}I-labeled fibrinogen (pooled) were introduced into the subject‟s circulation. The graft segment was primed with isotonic saline and added into the shunt circuit. Blood flow through the circuit was controlled with a single distal clamp and continuously monitored to be ~100 ml min^{-1}. Platelet accumulation in the central 20 mm of the graft was measured every three minutes for one hour by scintigraphy using a gamma camera. After the one-hour exposure, the graft segment was removed from the shunt circuit and flushed with isotonic saline until all visible blood was removed. The silicone tubing on either side of the LP graft was then removed, and the LP graft was stored at 4 $^{\circ}$C for 30 days, allowing ^{111}In decay and quantification of ^{125}I-labelled fibrin/fibrinogen deposits on the central 50 mm of each LP graft with a gamma camera.

Platelet and fibrin deposition on the LP grafts were compared to historical data from ePTFE grafts (n=6) of similar size, using t-tests at 15, 30, 45, and 60 min. A p-value of 0.05 was considered statistically significant.

This preclinical study was performed in accordance with the NIH Guide for the Care and Use of Laboratory Animals, which includes approval by the test facility IACUC. The test facility was registered with the USDA (#92-R-001) and accredited by AAALAC (aaalac.org).

In vivo biostability in a rabbit model: Pellethane 2363-80A (Lubrizol), Pellethane 2363-55D (Lubrizol) and LP polymer solutions with 25% (w/w) solids content in DMAc were applied to a glass plate using a film casting knife. DMAc was evaporated in a 55 °C oven overnight. LP films were peeled off the glass plates under running RO water. Final film thicknesses were determined to be 0.5-0.7 mm. Samples were defined as:

Positive Control: Pellethane 80A PEU (in vivo degradation well documented)[4,19]

Negative Control: Pellethane 55D PEU (strong history of clinical use, acceptable biostability) [4,19]

Test Article: LP SiPUU

Dumbbells of 3 cm length with holes in each end were punched from the sheets. Polycarbonate stretching mandrels were machined from sheet material. Dumbbell samples and mandrels were sterilized by ethylene oxide.

Under aseptic conditions, all polymer samples were strained with forceps and mounted onto polycarbonate mandrels, placing the sample end holes over the ends of the mandrels (**Figure 7**). Mandrel dimensions were carefully controlled to result in a 150% strain condition in the central, reduced cross section region of the dumbbell.

Strained samples were aseptically implanted subcutaneously above the dorsal muscle of rabbits. One of each sample type was implanted using random site selection on each side of the spine to eliminate position bias (Figure 7). A total of 8 rabbits and 16 of each sample type were implanted. Post-surgical health checks and wound site observations were performed daily.

Rabbits were euthanized after 12 weeks (n=4) or 24 weeks (n=4), when samples were carefully removed from the surrounding tissue. Samples were stored in normal saline until evaluation.

Previous reports indicate that visual and SEM surface assessments are the most sensitive method for detecting PU degradation in this type of study. Therefore, the surfaces of all

explanted samples were first evaluated visually using the scoring scheme as defined by Martin et al.[16,44]

1 = specimen smooth on all surfaces, no cracking observed

2 = patches of cracking or pitting over less than 10% of surface area

3 = patches of cracking or pitting over 10-70% of surface area

4 = fine cracking or pitting over greater than 70% of surface area

5 = any deep cracking covering less than 70% of surface area

6 = deep cracking covering more than 70% of surface area and/or specimen breakage.

Additionally, all explanted samples were evaluated using FE-SEM-EDAX (FEI Quantum 600) for evidence of degradation, such as pitting and cracking, and calcium deposition.

The in vivo portion of this biostability study was performed in Australia and approved by the Commonwealth Scientific and Industrial Research Organization (CSIRO) North Ryde Animal Ethics Committee in accordance with the Australian Code for the Care and Use of Animals for Scientific Purposes.

Statistical Analysis: Continuous variables are expressed as mean ± standard deviation. The statistical analysis of *ex vivo* platelet adhesion and thrombus formation was performed between the test and control groups, using a two-sided t-test in Microsoft Excel. Each group at each time point consisted of at least 5 data points. Data was used as collected, with no pre-processing. Significance was defined as $p \leq 0.05$.

Supporting Information

Supporting Information is available from the Wiley Online Library or from the author.

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Conflicts of Interest

All authors are either consultants to or employees of Foldax, Inc.

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Figures and Tables

Note that formal figures were submitted separately as individual files. The figures below are included for reviewers' reference only.

Figure 1. Foldax Tria Aortic Valve with LP leaflets. 23 mm outer diameter.

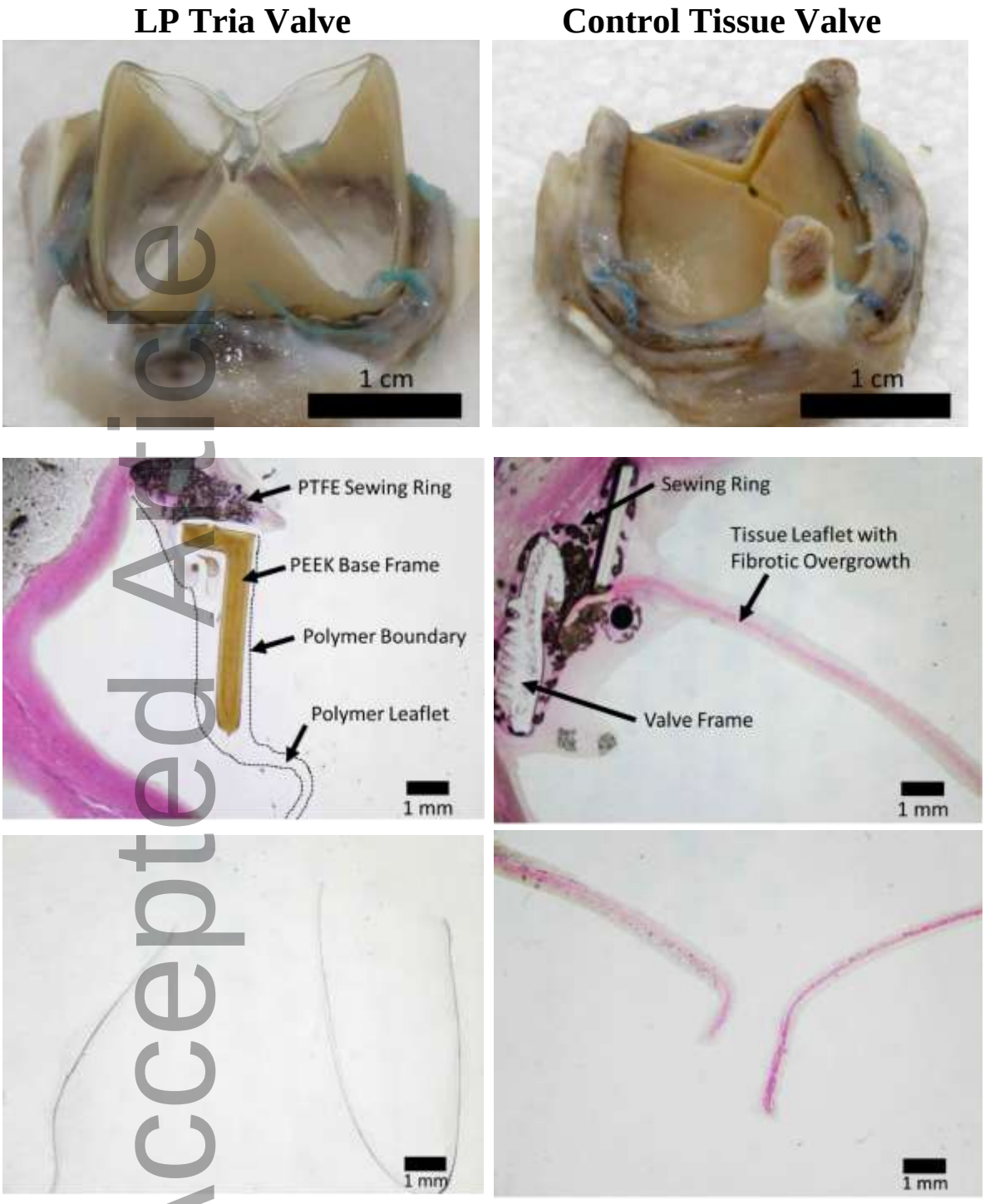


Figure 2. Representative 140 day ovine study explant pathology. Left: LP Tria valve images. Right: Carpentier-Edwards Perimount control tissue valve images. Top: Gross images after removal of valves and trimming of aortic tissue. Middle: Histopathology of frame, leaflet and surrounding tissue. Bottom: Histopathology of leaflet tips. All histopathology slides were prepared with H&E stain and are shown at a consistent magnification as demonstrated by the scale bars. Note that the LP material does not significantly absorb H&E stain, therefore the LP leaflets are difficult to visualize. The boundary of the LP material has been highlighted with a dashed line in the middle-left image.

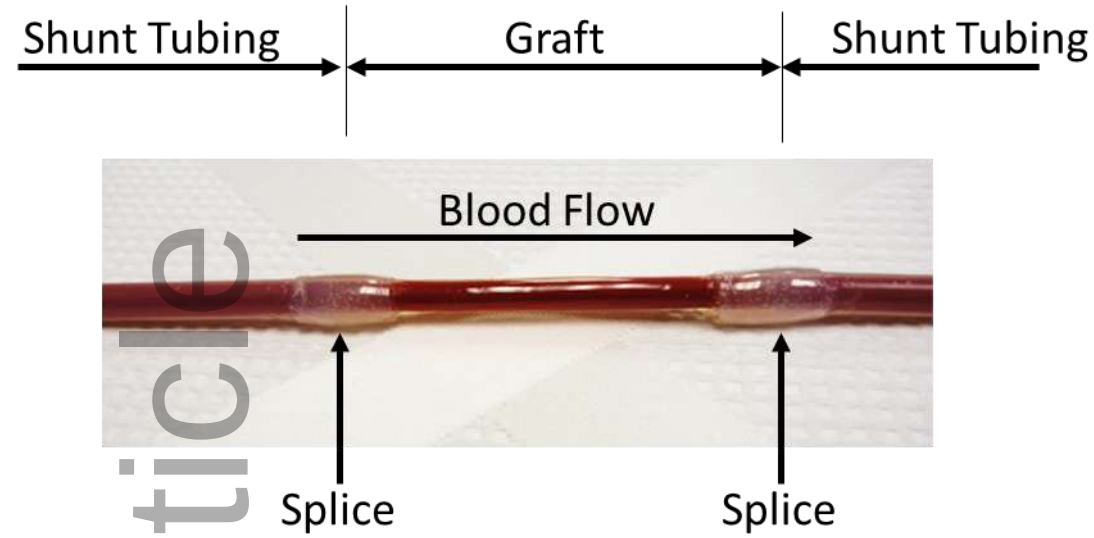


Figure 3. NHP blood circulating *ex vivo* through the LP graft (center) bonded to silicone rubber AV shunt tubing (left and right).

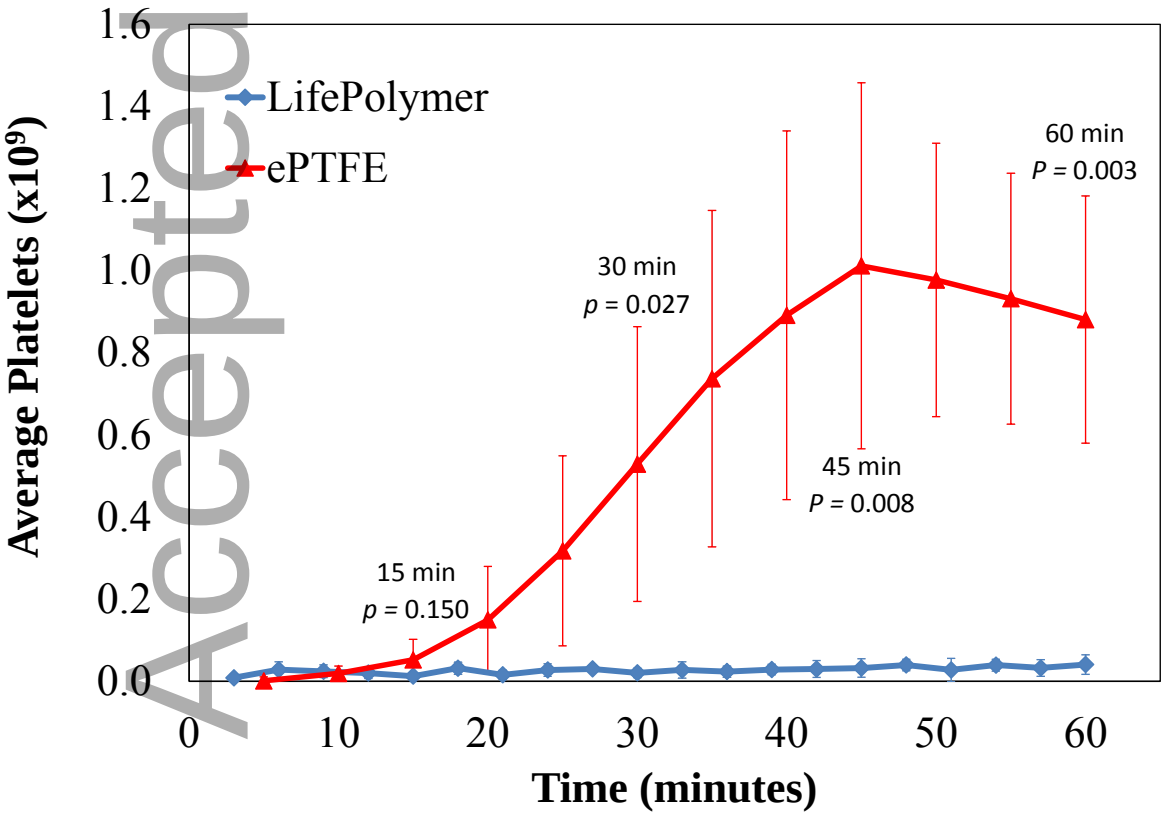


Figure 4. Platelet deposition on LifePolymer (LP) and ePTFE reference grafts in the NHP AV shunt loop. Data shown as average with error bars representing one standard deviation. *P* values represent a two-sided t-test performed between the LP test group (n=6) and the ePTFE reference group (n=5) at specific time points.

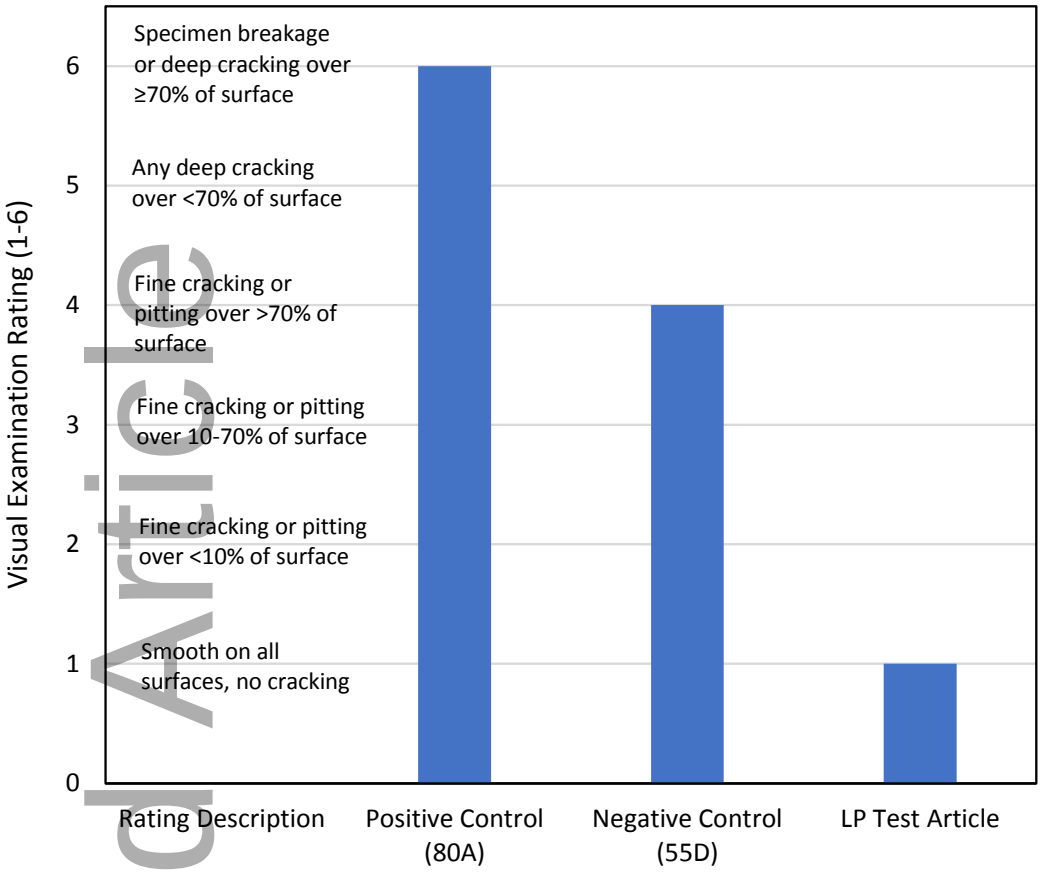
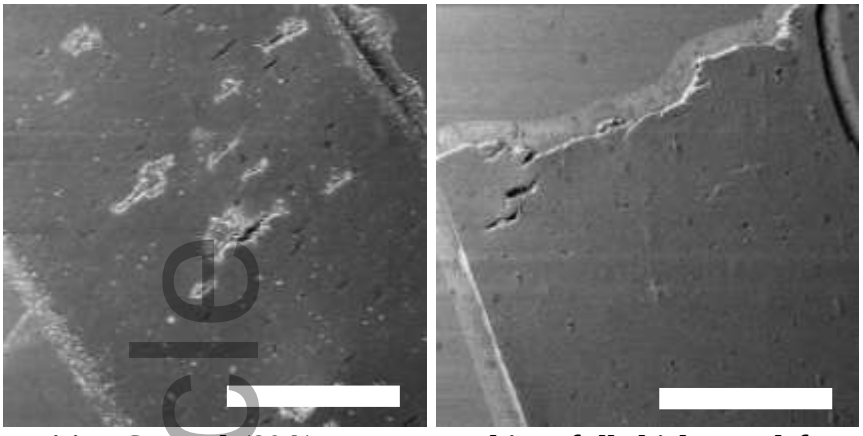
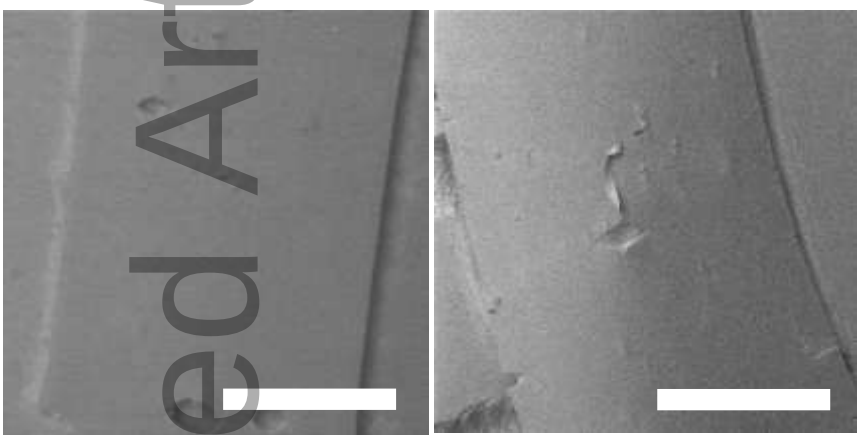


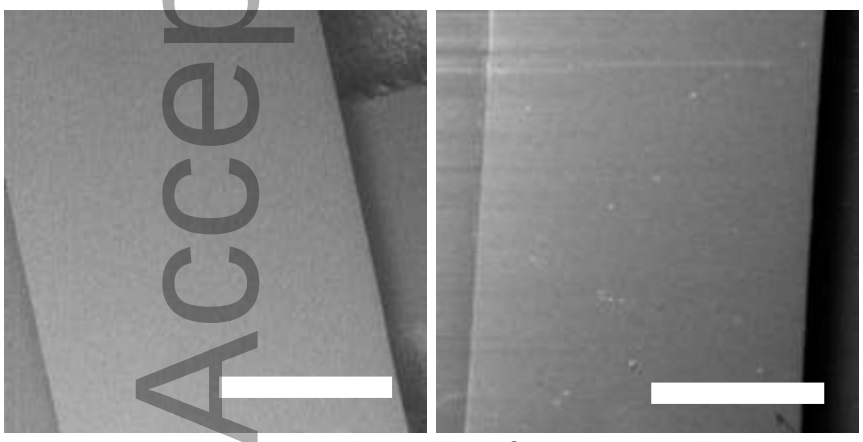
Figure 5. Assessment of degradation by visual examination of LP test article and PU control article cast films after 6 months *in vivo* under 150% strain. The definitions of each rating are provided on the left. The values shown in this graph represent an overall degradation assessment of each group.



Positive Control (80A): Deep cracking, full thickness defects, and specimen breakage.



Negative Control (55D): Minor surface pitting and cracking was observed on all samples.



LP Test Material: Smooth on all surfaces. No cracking.

Figure 6. Representative FE-SEM images of LP test article and PU control article cast films after 6 months *in vivo* under 150% strain. Images depict the center of two representative explanted dogbones from each group. Scale bars = 500 μm.

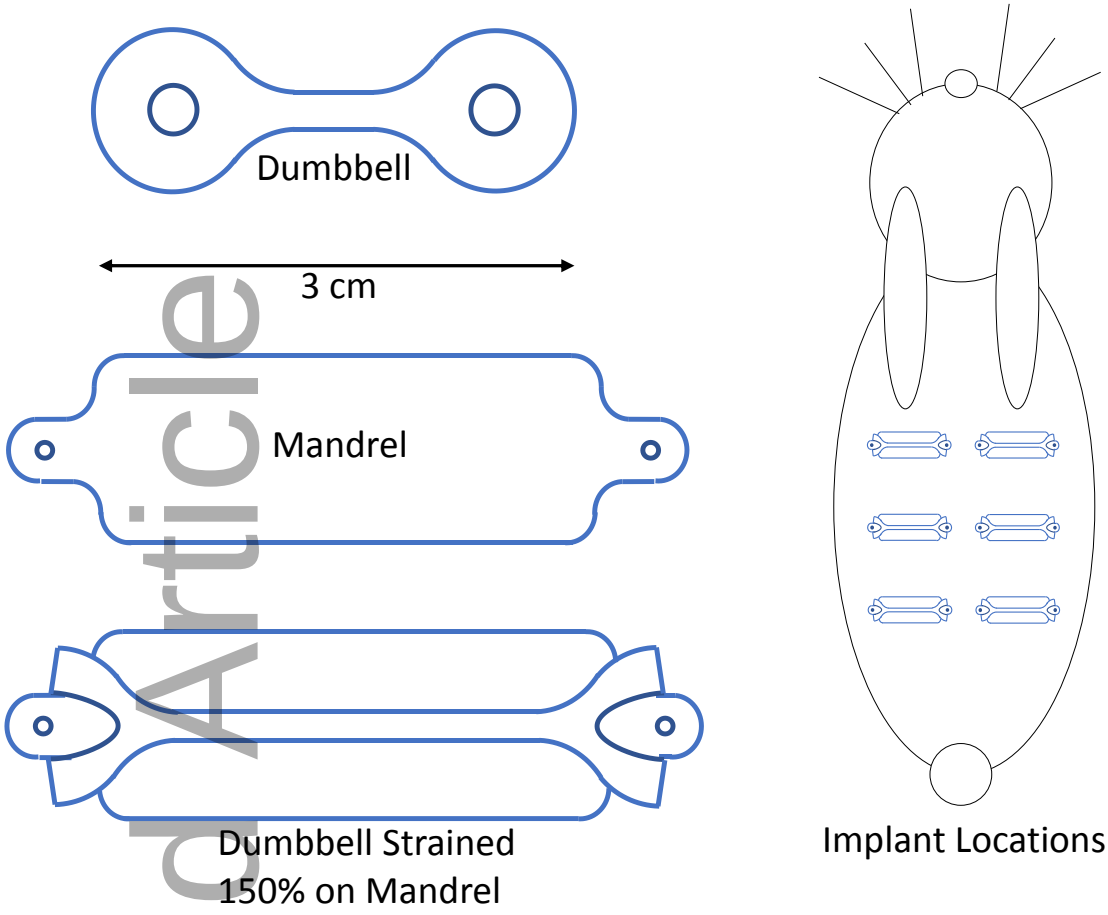


Figure 7. Rabbit biostability study sample construction and subcutaneous implant locations.

Table 1. SiPUU characterization data compiled from 14 commercial batches of LP.

Property	Analytical Tool	Unit	Average	StDev
M_w	GPC	Da	552,800	87,100
Mn	GPC	Da	175,600	21,300
PDI	GPC	n/a	3.15	0.31
T_g (initial heat)	DSC	°C	-4.0	1.7
T_g (cooling)	DSC	°C	-7.8	2.5
T_g (reheat)	DSC	°C	-3.6	2.0
IR Spectrum Correlation to Reference	ATR-FTIR	n/a	0.9977	0.0005
UTS	Tensile	MPa	38.3	1.8
Elongation	Tensile	%	760	43
Dynamic Modulus	Dynamic Tester	MPa	28.7	0.7
Tear Energy Threshold	Dynamic Tester	J m^{-2}	291	73

Table 2. ISO 10993 biocompatibility testing results on Tria valves with LP leaflets.

Test Description	Relevant Std	Results
Cytotoxicity - MEM Elution Assay	ISO 10993-5	Pass, Non-Cytotoxic. The positive and negative control results met the validity criteria. At 24, 48, and 72 hours, no reactivity, cell lysis, or reduction of cell growth was observed in the triplicate test article wells. Therefore, the test article received a grade of „0" and was considered Non-Cytotoxic.
Intracutaneous Reactivity Test	ISO 10993-10	Pass, Non-Irritant. All assay validity criteria were met. Erythema and edema was not observed at any site on any subject at any time point (24 h, 48 h, 72 h) for both normal saline extract and sesame seed oil extract. Therefore the test article was considered a non-irritant.
Acute Systemic Toxicity Test	ISO 10993-11	Pass, No Acute Systemic Toxicity. All 20 test and control animals remained in overall good health over the course of the 72 h study. None of the animals treated with the test article extracts showed a significantly greater reaction than the animals treated with the negative controls. The test article showed no evidence of acute systemic toxicity.
Sensitization - Guinea Pig Maximization	ISO 10993-10	Pass, Non-Sensitizer. All 36 test and control animals remained in overall good health over the course of the study. The overall pattern, intensity, and duration of reactions in the saline and sesame seed oil test article groups were identical to the negative control group, no erythema or edema observed. Based on the results of this study, the test article showed no evidence of delayed dermal contact sensitization and was considered a non-sensitizer.
Pyrogenicity - Material Mediated	ISO 10993-11	Pass, Non-Pyrogen. None of the 3 rabbits in this study showed a rise in body temperature >0.1 °C above its control temperature. Therefore, the test article showed no evidence of material mediated pyrogenicity.
Hemolysis Assay, Direct and Extract Methods	ISO 10993-4, ASTM F7756-13	Pass, Non-Hemolytic. Validity criteria for the positive and negative controls were met. The test

		article and test article extracts were determined to produce minimal hemolysis, equal to the negative controls. Therefore, the test articles were determined to be non-hemolytic using both direct and extract methods.
Partial Thromboplastin Time	ISO 10993-4, ASTM F2382	Pass, Test article performed better than the reference material (HDPE). The replicate variability of the controls, as well as the reference material, met the criteria for a valid assay. The test article had an average clotting time of 110.1 seconds, which was 112% of the negative control, and found to be statistically significant ($p<0.05$). Therefore, the statistical analysis indicates the test article performed better than the reference material.
Complement Activation	ISO 10993-4	Pass, Test article complement activation is lower than the negative reference material (HDPE). Using the SC5b-9 assay, the test article exhibited activation at 6394 ng mL^{-1} and was 0.1% of the normalized SC5b-9 concentration produced by the positive control, cobra venom factor (CVF). In comparison, the negative reference material (HDPE) exhibited 0.1% activation relative to the CVF. The test article results were statistically significantly ($p<0.05$) lower than the negative reference material.
Genotoxicity - AMES	ISO 10993-3	Pass, Non-Mutagenic. Under the conditions of this study, the mean number of revertant colonies for the test article was less than 2-fold over the mean number of revertant colonies for the negative control; therefore the test article was considered Non-Mutagenic.
Genotoxicity - Mouse Lymphoma	ISO 10993-3	Pass, Non-Mutagenic and Non-Clastogenic. The controls for the assay performed as required, qualifying both the assay and cell culture system as valid. The mutant frequencies and cloning efficiencies of preparations treated with test article were within the limits defined for a negative response. Accordingly, the test article is considered to be non-mutagenic and non-clastogenic.

Table 3. Quantity of leachable and extractable compounds from Tria valves Identified by GC-MS and LC-MS.

		Exhaustive Extraction			Leachables	
Analysis Type	**Compounds Identified**	**Water**	**50% Ethanol/ Water**	**Hexane**	**PBS**	**40% Ethanol/ Water**
GC-MS	Total	0	24	56	3	8
	Unique	0	11	12 [a)]	3	6
	Related Groups	0	5	4	0	1
LC-MS	Total	13 [b)]	91	72	9	109
	Unique	3 [b)]	8	2	7	17
	Related Groups	4	7	4	1	9

[a)] additional two compounds identified in hexane also identified in ethanol/water extract
[b)] two of the three compounds were identified by UV detection, not MS detection

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A siloxane poly(urethane-urea) elastomer recently developed for heart valve leaflets demonstrates a robust, scalable synthesis process with minimal variation. Aggressive testing confirms biocompatibility, negligible toxiciological risk from extractable and leachable compounds, low thrombogenicity, and excellent biostability. Ovine implantation of heart valves integrating this new elastomer confirms leaflet function and tissue/blood compatibility.

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