Degradation and *in vivo* evaluation of polycaprolactone, poly(ϵ -caprolactone-co-L-lactide), and poly-L-lactic acid as scaffold sealant polymers for murine tissue-engineered vascular grafts

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Aim: This study evaluates scaffold degradation and neotissue formation as a function of sealant polymer composition in tissue-engineered vascular grafts (TEVGs). **Materials & methods:** Scaffolds fabricated from polyglycolic acid core and sealant composed of polycaprolactone (PCL), poly-L-lactic-acid (PLLA) or 50:50 copolymer poly(ϵ -caprolactone-co-L-lactide) (PCLA) were analyzed *in vitro* using accelerated degradation and scanning electron microscopy, and *in vivo* following implantation in a murine inferior vena cava interposition model. **Results:** *In vitro* and *in vivo* characterization revealed statistically greater degradation of PCLA compared with both PCL and PLLA scaffolds, with similar neotissue formation across all groups. The wall thickness of PLLA TEVGs was statistically greater than PCL TEVGs at 2 weeks postimplant. **Conclusion:** Results of this study can be used to inform the rational design of future TEVGs.

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Much work has been done over the past two decades to engineer cardiovascular tissue for use in various reconstructive procedures [1]. Our group has specifically focused on developing tissue-engineered vascular grafts (TEVG), which grow, remodel and self-repair *in vivo*, which are particularly well suited for pediatric populations that would otherwise require re-operative procedures upon outgrowing synthetic grafts [2].

Though successes in the development of TEVGs were predominantly realized via trial-and-error approaches, a more time- and cost-efficient approach must be adopted. Our work has therefore shifted to include the use of computational modeling to rationally optimize scaffold design. Computational models have been developed to predict salient features of vascular growth and remodeling (G&R) in response to perturbations of biomechanical stimuli following disease or injury. G&R models for native vessels were recently extended to predict the evolution of both arterial TEVGs cultured in bioreactors and venous interposition TEVGs *in vivo* [3,4]. These models can be informed by a series of *in vivo* experiments testing the effects of extreme scaffold parameters on neovessel formation. Once sufficient experimental data are collected with which to generate the proposed model, parametric studies may be conducted to identify scaffolds that optimize the biomechanical resemblance of TEVGs to native vessels. To that effect, this study seeks to evaluate scaffold degradation as a function of sealant polymer composition, the data for which can later be used to inform our computational model.

The host inflammatory response, as well as biological function and mechanical integrity of neotissue, is strongly influenced by material and structural properties of the polymeric construct. If the scaffold degrades too quickly, TEVG dilation and subsequent rupture may occur. Alternatively, prolonged scaffold degradation may elicit a sustained foreign body reaction that leads to calcium deposition and increased TEVG stiffness. This results in

compliance mismatch with the native vessel and unfavorable changes in hemodynamics, which increase the risk of TEVG failure due to fibrosis or stenosis. Optimizing the rate of scaffold degradation is, therefore, critical to TEVG design [5].

Scaffolds, such as those designed by our group, are made of two biodegradable materials, an inner fiber layer that provides the majority of the initial mechanical stiffness of the scaffold, and a sealant layer that binds the fiber layer together, defines the shape of the scaffold and acts as the majority of the surface with which the body interacts. Since several properties of a scaffold are dominated by those of its outer sealant layer, modulating the sealant polymer composition would allow a simple and versatile means by which to alter degradation kinetics and thereby influence neotissue development, maintenance and remodeling. Poly(ϵ -caprolactone) (PCL) degrades at a slower rate than poly(L-lactic acid) (PLLA) [6]. We therefore characterized degradation rates of scaffolds fabricated from a poly(glycolic acid) (PGA) fiber core and sealed with an outer layer composed of either PCL, PLLA, or a 50:50 copolymer solution of poly(ϵ -caprolactone-co-L-lactide) (PCLA). Last, we used a murine inferior vena cava (IVC) interposition model to compare neotissue formation and cell infiltration between TEVGs. While PGA, PCL, PLLA and PCLA all have well-established biocompatibility in biomedical research [7,8], this work represents a direct head-to-head comparison using identical processing methods and *in vivo* study design, allowing a more direct comparison of the effects of the different polymer sealants.

Methods

Scaffold fabrication

Sealant polymer solutions were made by dissolving PCL, PLLA or PCLA at 5% by weight in 1,4-dioxane. Biodegradable polymeric scaffolds were fabricated using previously established methods [9]. Nonwoven PGA felt with a fiber diameter of 16 μ m was wrapped around a 20 G (*in vitro*) or 19 G (*in vivo*) needles to set the internal diameter. The needle and felt were fitted inside a 10 μ l pipette tip with a straight side wall to set the external diameter. The 40 μ l of sealant polymer solution was injected into the mold, and then the entire mold was frozen to -80°C and lyophilized overnight to remove the dioxane. Resulting scaffolds were trimmed to 3 mm and inspected to ensure consistent sealant coating and lack of defects.

Scanning electron microscopy

Scaffolds were cut, oriented to show luminal, adventitial and transverse views, and carbon taped to a scanning electron microscopy (SEM) mount. Samples were sputter coated with gold under vacuum to a thickness of 3 nm. SEM images were obtained using a Hitachi Scanning Electron Microscope (Hitachi Group, Tokyo, Japan) at 5 kV and 10,000 mA at 100 $\times$ and 500 $\times$.

In vitro scaffold accelerated degradation

Scaffolds ($n = 4$ for each group) were incubated in 1 ml 1 $\times$ phosphate-buffered saline (PBS; Thermo Fisher Scientific, MA, USA) heated to 50°C. This *in vitro* degradation model acts as an accelerated model of *in vivo* polymer degradation, which primarily occurs via temperature-dependent hydrolysis [10]. At end points (days 1, 2, 3, 4 and 5), scaffolds were removed from PBS, rinsed in distilled water and lyophilized overnight. Five days were chosen as the final examined end point for accelerated degradation as samples degraded past this point were not able to retain mechanical stability necessary for evaluation. Scaffolds were weighed after lyophilization and dry weights were compared with predegradation weights to determine percent mass remaining.

Implantation & harvest

All scaffold designs were examined by surgeons prior to implantation and found to have adequate and similar suture retention and handling, as well as the ability to withstand venous pressure within the mouse model. All animal protocols were approved and monitored by the Institutional Animal Care and Use Committee at Nationwide Children's Hospital. Scaffolds were sterilized via exposure to UV light. Using previously established sterile microsurgical techniques [9], scaffolds ($n = 5$ for each group) were implanted as IVC interposition grafts below the renal veins in 15 female, C57/BL6 mice (Jackson Labs, ME, USA) between 10 and 12 weeks of age. TEVG patency was assessed *in vivo* using a high frequency ultrasound system (VisualSonics, Ontario, Canada). TEVGs were harvested 2 weeks following implantation.

Figure 1. Scaffold characterization via scanning electron microscopy. Representative scanning electron microscopy images showing transverse (A–C) and luminal (D–F) sections of preimplant polycaprolactone (A & D), poly(ε-caprolactone-co-L-lactide) (B & E) and poly(L-lactic acid) (C & F) scaffolds. Scale bars = 500 μm (A–C) and 100 μm (D–F).

Histology & immunohistochemistry

Explanted TEVGs were fixed in formalin, embedded in paraffin, and serially sectioned from the center mm of the graft (4 μm thick). Sections were stained with hematoxylin and eosin (H&E) and Masson's trichrome (TRI). Unstained sections used for immunohistochemistry staining were deparaffinized, rehydrated and blocked for endogenous peroxidase activity and nonspecific background staining prior to a 30 min incubation with the following primary antibodies: rabbit anti-CD68 (1:2000; Abcam, Cambridgeshire, England), rabbit anti-CD31 (1:500; Abcam), rabbit anti-calponin (1:500; Abcam), rabbit anti-collagen III (1:1000; Abcam) or rabbit anti-collagen I (1:1000; Abcam). Primary antibody binding was detected via incubation with a goat antirabbit biotinylated secondary antibody (1:1500; Vector Laboratories, CA, USA), followed by incubation with Elite ABC-Peroxidase Reagent RTU (Vector Laboratories). Color development was achieved via chromogenic reaction with 3,3-diaminobenzidine. Nuclei were counterstained with hematoxylin.

Quantitative analysis

Surface pore size and porosity of scaffolds were calculated using ImageJ software (NIH, MD, USA). TEVG percent neotissue area (of total wall area), wall thickness (excluding surrounding connective tissue) and luminal diameter were quantified from H&E images at 5× using ImageJ software. Percent area of remaining scaffold material (of total wall area) was quantified from polarized TRI images at 5× using ImageJ software. Samples stained for TRI, CD68, CD31, calponin, collagen III or collagen I were imaged at four nonoverlapping locations at 20×. For an individual sample, ImageJ software was used to quantify percent area of positive calponin, TRI, collagen III or collagen I staining as an average across the four imaged locations. Macrophage infiltration (positive CD68 expression) and endothelialization (positive CD31 expression) were assessed qualitatively. Due to low sample sizes (n = 5 for each group), the single nonpatent TEVG in each group was excluded from histological analysis to avoid confounding factors.

Statistical analysis

Data are reported as mean ± standard deviation. Statistical differences between groups were determined using one-way analysis of variance (ANOVA), followed by Tukey's multiple comparison test. p-values < 0.05 were considered statistically significant.

Results

Scaffold characterization

SEM showed randomly organized fibers across all preimplant scaffolds and the following relationships between porosity: PCL < PCLA < PLLA (Figure 1). Specifically, PLLA scaffolds were shown to have a pore size of 14.2 ± 4.1 μm with a porosity of 87.7%, and the PCLA scaffolds had 13.3 ± 6.3 μm pores with 87.9% porosity. PCL scaffolds showed a nearly entirely smooth surface with very sparse pores, with a level of porosity and average pore size that were below the detectable level of the ImageJ software.

Figure 2. *In vitro* scaffold degradation. Percent mass remaining of preimplant PCL, PCLA, and PLLA scaffolds following heated degradation in 1X phosphate-buffered saline at 50°C. PCL: Poly(caprolactone); PCLA: poly(ϵ -caprolactone-co-L-lactide); PLLA: Poly-L-lactic-acid.

In vitro characterization of accelerated degradation showed a statistically significant variation in percent mass remaining at 5 days between preimplant PCL ($89.01 \pm 7.49\%$), PCLA ($56.61 \pm 6.38\%$) and PLLA ($77.66 \pm 3.15\%$) scaffolds (Figure 2; $p < 0.0001$). A *post-hoc* Tukey honestly significant difference (HSD) test showed statistically greater degradation of PCLA compared with both PCL ($p = 0.0010$) and PLLA ($p = 0.0019$) scaffolds. A statistically significant difference was not detected between PCL and PLLA scaffolds.

Animal survival

Graft occlusion was defined as 100% narrowing of the luminal diameter. Patency was defined as nonocclusion. Within each group, four out of five TEVGs were patent without thrombosis or aneurysm dilation.

Histology & immunohistochemistry

All histology was performed on the midgraft portion of the explants. H&E staining showed extensive cell infiltration within patent PCL, PCLA and PLLA TEVGs (Figure 3A–C). Percent neotissue area did not statistically differ between patent PCL ($10.68 \pm 4.41\%$), PCLA ($9.85 \pm 3.68\%$) and PLLA ($13.52 \pm 6.20\%$) TEVGs (Figure 3D; $p = 0.5581$). There was a statistically significant variation in wall thickness between patent PCL ($360.09 \pm 57.30 \mu\text{m}$), PCLA ($368.91 \pm 20.31 \mu\text{m}$) and PLLA ($440.36 \pm 27.97 \mu\text{m}$) TEVGs (Figure 3E; $p = 0.0317$). A *post-hoc* Tukey HSD test showed statistically greater wall thickness of PLLA compared with PCL TEVGs ($p = 0.0397$). Luminal diameter did not statistically differ between patent PCL ($1005.37 \pm 97.01 \mu\text{m}$), PCLA ($1112.93 \pm 79.44 \mu\text{m}$) and PLLA ($964.09 \pm 71.17 \mu\text{m}$) TEVGs (Figure 3F; $p = 0.0791$).

TRI staining (Figure 4A–C) observed with polarized light microscopy (Figure 4D–F) showed a statistically significant variation in percent area of remaining scaffold material between patent PCL ($8.56 \pm 0.68\%$), PCLA ($4.34 \pm 1.79\%$) and PLLA ($8.19 \pm 0.45\%$) TEVGs (Figure 4G; $p < 0.0009$). A *post-hoc* Tukey HSD test showed statistically greater degradation of PCLA compared with both PCL ($p = 0.0014$) and PLLA ($p = 0.0025$) TEVGs. A statistically significant difference was not detected between PCL and PLLA TEVGs. These relationships mirror those revealed from *in vitro* characterization of accelerated scaffold degradation.

Extensive infiltration by CD68⁺ macrophages in patent PCL, PCLA and PLLA TEVGs suggested an inflammatory-mediated process of regeneration (Figure 5A–C). A monolayer of endothelial cells (ECs), delineated by positive immunostaining for CD31, was evident along the luminal surface of patent PCL, PCLA and PLLA TEVGs (Figure 5D–F). Multiple layers of elongated and spindle-shaped contractile smooth muscle cells (SMCs), delineated by positive immunostaining for calponin, were circumferentially organized external to the EC monolayer of patent PCL, PCLA and PLLA TEVGs (Figure 5G–I). Percent contractile SMC area did not statistically differ between patent PCL ($2.50 \pm 1.65\%$), PCLA ($3.83 \pm 0.123\%$) and PLLA ($3.37 \pm 1.58\%$) TEVGs ($p = 0.4726$).

TRI staining showed extensive total collagen deposition throughout the wall of patent PCL, PCLA and PLLA TEVGs (Figure 6A–C). Immature and mature collagen were further characterized via immunostaining for collagen III (Figure 6D–F) and collagen I (Figure 6G–I), respectively. Immunohistochemistry staining showed increased collagen deposition surrounding residual scaffold material. Percent areas of total ($p = 0.2067$), immature ($p = 0.4195$)

Figure 3. Histological assessment of vascular neotissue formation. Representative images (scale bar = 200 μm) for hematoxylin and eosin staining of PCL (A), PCLA (B) and PLLA (C) tissue-engineered vascular grafts 2 weeks postimplantation. Percent neotissue area (D), wall thickness (E) and luminal diameter (F) of patent PCL, PCLA and PLLA tissue-engineered vascular grafts 2 weeks postimplantation (n = 4 for all groups).

Data in graphs are expressed as mean ± standard deviation; *p < 0.05.

PCL: Poly(caprolactone); PCLA: Poly(ε-caprolactone-co-L-lactide); PLLA: Poly-L-lactic-acid.

and mature (p = 0.9072) collagen did not statistically differ between patent PCL, PCLA and PLLA TEVGs (Figure 6J).

Discussion

We have previously demonstrated in our murine TEVG models that neotissue development, maintenance and remodeling are dependent upon initiation of an immunomodulatory cytokine cascade, EC and SMC recruitment from adjacent vessels, and extracellular matrix production [11]. Optimizing the rate of scaffold degradation is critical to ensuring adequate, but not excessive, activation of the above processes. If the scaffold is present for too long, a prolonged foreign body response ensues, leading to calcification and increased stiffness of the construct that may shield the TEVG from biomechanical stimuli necessary to ensure appropriate neotissue formation [12].

The mechanisms of degradation for PCL, PLLA and their copolymers are qualitatively similar, though PCL degrades at a slower rate than PLLA due to relatively increased hydrophobicity and crystallization [13]. Degradation occurs as a two-stage process including random, nonenzymatic hydrolytic cleavage of ester bonds; and internal autocatalysis via newly generated carboxyl and hydroxyl end groups [14]. This degradation process seen in the literature can be confirmed in our model by future studies utilizing size exclusion chromatography (SEC) and gel permeation chromatography (GPC) techniques. We hypothesized the following relationships between scaffold degradation rates: PCL < PCLA < PLLA. However, *in vitro* and *in vivo* characterization of degradation revealed statistically greater degradation of PCLA compared with both PCL and PLLA scaffolds.

It has been shown that within a PLLA composition of 40%, the degradation rate of PCL/PLLA copolymers remains intermediate to that of either homopolymer and increases with PLLA content [15]. However, at PLLA compositions >40%, the rate of copolymer degradation exceeded that of either homopolymer [16]. We propose that copolymerization of PCL and PLLA for the fabrication of PCLA scaffolds affected the first stage of degradation by altering rates of hydration and hydrolysis. The second stage of degradation is defined by bimodal molecular weight distribution across a polymer. The accumulation of carboxyl and hydroxyl end group by-products accelerates the rate of internal degradation relative to the outer surface. Once small enough, water-soluble monomers and oligomers resulting from internal degradation diffuse out and promote degradation of the comparatively higher

Figure 4. Histological assessment of *in vivo* scaffold degradation. Representative brightfield (A–C) and polarized (D–F) images (scale bar = 20 μm) for trichrome staining of PCL (A & D), PCLA (B & E) and PLLA (C & F) tissue-engineered vascular grafts 2 weeks postimplantation, (G) Percent area of remaining scaffold material in patent PCL, PCLA and PLLA tissue-engineered vascular grafts 2 weeks postimplantation (n = 4 for all groups). Data in graph are expressed as mean ± standard deviation.

*p < 0.05.

PCL: Poly(caprolactone); PCLA: Poly(ε-caprolactone-co-L-lactide); PLLA: Poly-L-lactic-acid.

molecular weight outer surface. Therefore, we propose that the second stage of degradation was affected by changes in molecular weight distribution following copolymerization [14]. These results encourage investigation of PCLA scaffolds with PLLA compositions <40%. Alternative methods of modulating scaffold degradation kinetics include fabricating sealant layers from any of the myriad of biocompatible polymers available [17]; and adjusting graft parameters to modify fiber diameter or porosity, which strongly influence degradation rate by controlling the available surface area for scaffold–cell interaction [5].

The wall thickness of patent PLLA TEVGs was statistically greater than patent PCL TEVGs (Figure 3E) at 2 weeks postimplant, despite consistent fabrication methods and preimplant thickness across all TEVGs. We have previously demonstrated in a murine IVC interposition model that early stage stenosis is characterized by narrowing of the lumen diameter due to thickening of the TEVG wall [18]. However, luminal diameter did not statistically vary between groups (Figure 3F). Additionally, staining of PLLA TEVGs showed some separation of the neotissue layer from the adjacent scaffold, which may have falsely increased the measured wall thickness (Figure 3C). We therefore propose that thickening of the wall in patent PLLA TEVGs is not an indicator of early stage stenosis.

Figure 5. Histological characterization of macrophages, endothelial cells and contractile smooth muscle cells. Representative images (scale bar = 20 μ m) for CD68 (A–C), CD31 (D–F) and calponin (G–I) immunostaining of PCL (A, D & G), PCLA (B, E & H) and PLLA (C, F & I) tissue-engineered vascular grafts 2 weeks postimplantation. PCL: Poly(caprolactone); PCLA: Poly(ϵ -caprolactone-co-L-lactide); PLLA: Poly-L-lactic-acid.

Follow-up studies include mechanical testing of patent PCL, PCLA and PLLA TEVGs to determine whether any differences or similarities in wall thickness translate to changes in TEVG performance.

At 2 weeks postimplant, endothelialization, contractile SMC accumulation and collagen deposition were similar in patent PCL and PLLA TEVGs compared with previously validated PCLA TEVGs. The formation of well-organized neotissue without thrombosis or aneurysmal dilation demonstrates that PCL, PCLA and PLLA TEVGs underwent successful remodeling. Follow-up studies are necessary to include the investigation of scaffolds with a broader range of degradation profiles.

Due to the effect of the sealant polymers' properties, scaffold porosity was altered in tandem with the sealant polymer composition, despite undergoing identical manufacturing methods. It has been shown that scaffold degradation kinetics are strongly influenced by fiber alignment and diameter, pore size and porosity [5]. This effect allowed for an interplay between changes in porosity, degradation rate and TEVG wall thickness. Continued careful characterization of scaffold parameters in follow-up studies will allow us to comprehensively evaluate the individual effects of these interconnected variables. Chemical evaluation techniques such as gel permeation chromatography and size exclusion chromatography in future studies may allow for more detailed description and evaluation of the degradation profiles of the scaffold polymers.

Our previous results have shown that TEVG performance at 2 weeks strongly correlates with long-term outcomes [19,20]. Although macrophage, EC, contractile SMC and collagen recruitment have been shown to peak 2 weeks postimplantation, neotissue formation is difficult to evaluate at short-term follow-up due to remaining scaffold material [18]. In follow-up studies, neotissue formation can be monitored until scaffold degradation is complete to allow assessment of long-term patency rates, as well as events such as total collagen normalization (4 weeks) and calcification (6 months) [21].

Perhaps the most important aspect of this study is its implication for the improved understanding and manipulation of scaffold degradation profiles. Data from this pilot study can later be used to guide the design of scaffolds with varying degradation kinetics and evaluate their effect on the biomechanical properties of neotissue. This information can then be used to inform our computational model for TEVG evolution.

Figure 6. Collagen visualization and quantification. Representative images (scale bar = 20 μ m) for trichrome (A–C), collagen III immunohistochemistry (D–F) and collagen I immunohistochemistry (G–I) staining of polycaprolactone (A, D & G), PCLA (B, E & H) and PLLA (C, F & I) tissue-engineered vascular grafts 2 weeks postimplantation, (J) Percent areas of total collagen, delineated by positive trichrome staining, immature collagen, delineated by positive immunostaining for collagen III, and mature collagen, delineated by positive immunostaining for collagen I, in patent PCL, PCLA and PLLA tissue-engineered vascular grafts 2 weeks postimplantation (n = 4 for all groups). Data in graph are expressed as mean $\pm$ standard deviation.

*p < 0.05.

PCL: Poly(ϵ -caprolactone); PCLA: Poly(ϵ -caprolactone-co-L-lactide); PLLA: Poly-L-lactic-acid.

Conclusion

In our study, we demonstrated how altering the sealant polymer composition can affect the rate of scaffold degradation *in vitro* and *in vivo*, as well as alter TEVG wall thickness. The results of this and follow-up studies aimed at comparing the biomechanical performance of scaffolds fabricated with different sealants can be used to validate and extend our computational model for neovessel formation, leading to the more refined development of future TEVGs.

Translational perspective

There have been tremendous advances in developing TEVGs, which grow, remodel and self repair *in vivo*. Implantation of biodegradable polymeric scaffolds has shown particular promise in both animal and clinical studies [1,2].

Attention initially focused on survivability at implantation and avoiding acute thrombosis, but has shifted toward optimizing the long-term biomechanical stability and growth potential of TEVGs. G&R computational models, used to evaluate the G&R of native vasculature in response to biomechanical stimuli, have recently been extended to predict the evolution of both arterial TEVGs cultured in bioreactors and venous interposition TEVG's *in vivo*. These models rely on information gathered from *in vivo* experiments testing the effects of extreme scaffold parameters on neovessel formation [3,4]. Parameters with demonstrated importance in influencing structure–function relationships and the host inflammatory response include scaffold degradation rate, pore size, porosity, fiber diameter and alignment, and axial stiffness [5]. This study evaluated scaffold degradation as a function of sealant polymer composition, and our results can be used to inform a novel computational model that nonintrusively incorporates concepts of parameter sensitivity, optimization, nondimensionalization and uncertainty quantification within a validated G&R model. Once sufficient experimental data are collected with which to generate the proposed model, parametric studies may be conducted to identify scaffolds that optimize the biomechanical resemblance of TEVGs to native vessels. Promising scaffold designs can subsequently be fabricated and evaluated *in vivo*. Development of the proposed model would ultimately allow for rational TEVG design *in silico*, prior to resource-intensive testing *in vivo*.

In the USA, congenital cardiac anomalies are a leading cause of neonatal mortality. Notwithstanding the successes of synthetic vascular grafts for replacement conduits, a significant limitation is the need for re-operative procedures due to the inability of synthetic grafts to grow with a pediatric patient. In addition to having a decreased risk of rejection, infection and calcific degradation compared with their synthetic counterparts, TEVGs are well suited for the pediatric population because they can grow and remodel *in vivo* [1,2]. By transitioning from trial and error experimental approaches to *in silico* optimization of TEVG design, we will be able to more effectively and efficiently progress toward clinical application.

Summary points

- Optimizing the rate of scaffold degradation is critical to tissue-engineered vascular graft (TEVG) design because rapid scaffold degradation may lead to TEVG dilation and subsequent rupture, while prolonged scaffold degradation may elicit a sustained foreign body reaction that leads to calcium deposition and increased TEVG stiffness.
- Since the properties of a scaffold are dominated by those of its outer layer, modulating the sealant polymer composition allows a simple and versatile mechanism of altering degradation kinetics, and thereby influencing neotissue development, maintenance and remodeling.
- Scaffolds fabricated from a poly(glycol acid) core and sealant composed of poly(caprolactone) (PCL), poly(L-lactic acid) (PLLA) or 50:50 copolymer (poly[ε-caprolactone-co- L-lactide] [PCLA]) were analyzed *in vitro* using accelerated degradation and scanning electron microscopy, and *in vivo* following implantation in a murine inferior vena cava interposition model.
- Because the mechanisms of degradation for PCL, PLLA and their copolymers are qualitatively similar and PCL degrades at a slower rate than PLLA, we hypothesized the following relationships between scaffold degradation rates: PCL < PCLA < PLLA.
- *In vitro* and *in vivo* characterization revealed statistically greater degradation of PCLA compared with both PCL and PLLA scaffolds, with similar neotissue formation across all groups.
- The wall thickness of PLLA TEVGs was statistically greater than PCL TEVGs at 2 weeks postimplant, but we propose that PLLA TEVG wall thickening is not an indicator of early-stage stenosis because luminal diameter, which has been shown to narrow in early-stage stenosis, did not statistically vary between groups.
- It has been shown that the degradation rate of PCL/PLLA copolymers remains intermediate to that of either homopolymer and increases with PLLA content within a PLLA composition of 40%, but exceeds that of either homopolymer at PLLA compositions >40%.
- We propose that copolymerization of PCL and PLLA for the fabrication of PCLA scaffolds affected the first stage of degradation by altering rates of hydration and hydrolysis, and affected the second stage of degradation by influencing molecular weight distribution.
- Key follow-up studies include the continued careful characterization of scaffold parameters that have been shown to influence scaffold degradation, such as fiber alignment and diameter, pore size, and porosity, in order to comprehensively evaluate the individual effects of interconnected variables.
- The results of this and follow-up studies aimed at comparing the biomechanical performance of scaffolds with varying degradation kinetics can be used to validate and extend our computational model for neovessel formation, leading to the rational development of future TEVGs.

Author contributions

The authors certify that each co-author listed above participated sufficiently in the work to take responsibility for the content, and that all those who qualify are listed. Authorship credit was based on substantial contributions to the conception or design of the work; or the acquisition, analysis or interpretation of data for the work; and drafting the work or revising it critically for important intellectual content; and final approval of the version to be published; and agreement to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

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Ethical conduct of research

All animal protocols were approved and monitored by the Institutional Animal Care and Use Committee at Nationwide Children's Hospital.

References

Papers of special note have been highlighted as: ● of interest; ●● of considerable interest

1. Pashneh-Tala S, Macneil S, Claeysens F. The tissue-engineered vascular graft-past, present, and future. *Tissue Eng. Part B Rev.* 22(1), 68–100 (2015).
2. Best C, Onwuka E, Pepper V, Sams M, Breuer J, Breuer C. Cardiovascular tissue engineering: preclinical validation to bedside application. *Physiology (Bethesda)* 31(1), 7–15 (2016).
3. Udelsman BV, Khosravi R, Miller KS *et al.* Characterization of evolving biomechanical properties of tissue engineered vascular grafts in the arterial circulation. *J. Biomech.* 47(9), 2070–2079 (2014).
4. Miller KS, Lee YU, Naito Y, Breuer CK, Humphrey JD. Computational model of the *in vivo* development of a tissue engineered vein from an implanted polymeric construct. *J. Biomech.* 47(9), 2080–2087 (2014).
5. Miller KS, Khosravi R, Breuer CK, Humphrey JD. A hypothesis-driven parametric study of effects of polymeric scaffold properties on tissue engineered neovessel formation. *Acta Biomater.* 11, 283–294 (2015).
- **Identifies six, tractable and fundamental polymeric scaffold parameters to be used in a computational model evaluating graft biomechanical function *in vivo*.**
6. Chu B, Zhang L, Qu Y *et al.* Synthesis, characterization and drug loading property of monomethoxy-poly(ethylene glycol)-poly(epsilon-caprolactone)-poly(D,L-lactide) (MPEG-PCLA) copolymers. *Sci. Rep.* 6, 34069 (2016).
7. Dean EW, Udelsman B, Breuer CK. Current advances in the translation of vascular tissue engineering to the treatment of pediatric congenital heart disease. *Yale J. Biol. Med.* 85(2), 229–238 (2012).
8. Ulerly BD, Nair LS, Laurencin CT. Biomedical applications of biodegradable polymers. *J. Polym. Sci. B Polym. Phys.* 49(12), 832–864 (2011).
9. Roh JD, Nelson GN, Brennan MP *et al.* Small-diameter biodegradable scaffolds for functional vascular tissue engineering in the mouse model. *Biomaterials* 29(10), 1454–1463 (2008).
10. Lyu S, Untereker D. Degradability of polymers for implantable biomedical devices. *Int. J. Mol. Sci.* 10(9), 4033–4065 (2009).
11. Roh JD, Sawh-Martinez R, Brennan MP *et al.* Tissue-engineered vascular grafts transform into mature blood vessels via an inflammation-mediated process of vascular remodeling. *Proc. Natl Acad. Sci. USA* 107(10), 4669–4674 (2010).
- **Describes the inflammatory mechanism of vascular remodeling following implantation of scaffolds seeded with autologous bone marrow mononuclear cells.**
12. Khosravi R, Best CA, Allen RA *et al.* Long-term functional efficacy of a novel electrospun poly(glycerol sebacate)-based arterial graft in mice. *Ann. Biomed. Eng.* 44(8), 2402–2416 (2016).
13. Pitt CG, Gratzl MM, Kimmel GL, Surles J, Schindler A. Aliphatic polyesters II. The degradation of poly (DL-lactide), poly (epsilon-caprolactone), and their copolymers *in vivo*. *Biomaterials* 2(4), 215–220 (1981).
14. Woodruff MA, Hutmacher DW. The return of a forgotten polymer-polycaprolactone in the 21st century. *Prog. Polym. Sci.* 35(10), 1217–1256 (2010).
- **Details the two-stage biodegradation of poly(caprolactone) (PCL), which is qualitatively similar to that of poly(L-lactic acid) and PCL/poly(L-lactic acid) copolymers.**

15. Ye WP, Du FS, Jin WH, Yang JY, Xu Y. *In vitro* degradation of poly(caprolactone), poly(lactide) and their block copolymers: influence of composition, temperature and morphology. *React. Funct. Polym.* 32(2), 161–168 (1997).
- **Characterizes *in vitro* degradation rates of PCL, polylactic acid (PLA) and their block copolymers as a function of polymer composition and temperature.**
16. Huang M, Li S, Huttmacher DW, Coudane J, Vert M. Degradation characteristics of poly(ϵ -caprolactone)-based copolymers and blends. *J. Appl. Polym. Sci.* 102(2), 1681–1687 (2006).
- **Characterizes *in vitro* improvements in degradability and hydrophilicity of PCL following copolymerization with polyethylene glycol or PLA.**
17. Chen Q, Liang S, Thouas GA. Elastomeric biomaterials for tissue engineering. *Prog. Polym. Sci.* 38(3–4), 584–671 (2013).
18. Hibino N, Yi T, Duncan DR *et al.* A critical role for macrophages in neovessel formation and the development of stenosis in tissue-engineered vascular grafts. *FASEB J.* 25(12), 4253–4263 (2011).
- **Defines early stage stenosis in grafts following scaffold implantation.**
19. Hibino N, Mejias D, Pietris N *et al.* The innate immune system contributes to tissue-engineered vascular graft performance. *FASEB J.* 29(6), 2431–2438 (2015).
20. Lee YU, De Dios Ruiz-Rosado J, Mahler N *et al.* TGF- β receptor 1 inhibition prevents stenosis of tissue-engineered vascular grafts by reducing host mononuclear phagocyte activation. *FASEB J.* 30(7), 2627–2636 (2016).
21. Fukunishi T, Best CA, Sugiura T *et al.* Tissue-engineered small diameter arterial vascular grafts from cell-free nanofiber PCL/chitosan scaffolds in a sheep model. *PLoS ONE* 11(7), e0158555 (2016).