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The degradation and performance of electrospun supramolecular vascular scaffolds examined upon in vitro enzymatic exposure

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Abstract

To maintain functionality during in situ vascular regeneration, the rate of implant degradation should be closely balanced by neo-tissue formation. It is unknown, however, how the implant’s functionality is affected by the degradation of the polymers it is composed of. We therefore examined the macro- and microscopic features as well as the mechanical performance of vascular scaffolds upon in vitro enzymatic degradation. Three candidate biomaterials with supramolecularly interacting bis-urea (BU) hard blocks (‘slow-degrading’ polycarbonate-BU (PC-BU), ‘intermediate-degrading’ polycarbonate-ester-BU (PC(e)-BU), and ‘fast-degrading’ polycaprolactone-ester-BU (PCL-BU)) were synthesized and electrospun into microporous scaffolds. These materials possess a sequence-controlled macromolecular structure, so their susceptibility to degradation is tunable by controlling the nature of the polymer backbone. The scaffolds were incubated in lipase and monitored for changes in physical, chemical, and mechanical properties. Remarkably, comparing PC-BU to PC(e)-BU, we observed that small changes in macromolecular structure led to significant differences in degradation kinetics. All three scaffold types degraded via surface erosion, which was accompanied by fiber swelling for PC-BU scaffolds, and some bulk degradation and a collapsing network for PCL-BU scaffolds. For the PC-BU and PC(e)-BU scaffolds this resulted in retention of mechanical properties, whereas for the PCL-BU scaffolds this resulted in stiffening. Our in vitro study demonstrates that vascular scaffolds, electrospun from sequence-controlled supramolecular materials with varying ester contents, not only display different susceptibilities to degradation, but also degrade via different mechanisms.

Statement of Significance

One of the key elements to successfully engineer vascular tissues in situ, is to balance the rate of implant degradation and neo-tissue formation. Due to their tunable properties, supramolecular polymers can be customized into attractive biomaterials for vascular tissue engineering. Here, we have exploited this tunability and prepared a set of polymers with different susceptibility to degradation. The polymers, which were electrospun into microporous scaffolds, displayed not only different susceptibilities to degradation, but also obeyed different degradation mechanisms. This study illustrates how the class of supramolecular polymers continues to represent a promising group of materials for tissue engineering approaches.

1. Introduction

There is a large clinical demand for small-diameter vascular conduits to treat patients suffering from cardiovascular disease or end-stage kidney disease [1,2]. Vascular conduits are required for coronary artery bypass grafting, for lower limb revascularization procedures, and for use in arteriovenous access shunts that are applied for hemodialysis. To date, the golden standard for arte-
rial replacement remains the use of the patient’s own vasculature (e.g., internal thoracic artery, radial artery or great saphenous vein), mainly because the tissue is biocompatible, has matching mechanical properties, and possesses a non-thrombogenic endothelium. Despite these advantages, autologous replacement is often not an option, because many patients lack appropriate vessels due to vascular disease or previous harvest. As a result, there is a growing demand for alternative vascular substitutes. However, currently available small-diameter vascular substitutes (>6 mm) have been characterized by poor biocompatibility, thrombosis, and intimal hyperplasia leading to stenosis [3–5]. To overcome these problems and address the need for small-diameter vascular conduits, tissue engineering (TE) approaches are being investigated to offer an alternative.

Various approaches for vascular TE using cell-laden or acellular biodegradable scaffolds (either of biological or synthetic origin) have been widely explored [6]. Recent developments have led to an increased focus on in situ TE using cell-free synthetic biodegradable scaffolds, also because this approach represents a clinically appealing strategy due to the off-the-shelf availability of implant materials [7]. In situ TE largely depends on the host’s capacity to colonize and populate the scaffold with endogenous cells that produce extracellular matrix (ECM). It also requires scaffolds that have sufficient strength to take over artery functionality immediately upon implantation, thereby withstanding the high mechanical demands imposed by the arterial high-pressure circulation. Furthermore, maintenance of the mechanical integrity during the build-up of neo-tissue by the host, while the scaffold is being degraded, is essential to avoid graft failure [8]. Hence, the scaffold must degrade in pace with neo-tissue formation to allow for a safe and mechanically stable transition from scaffold implant to living autologous blood vessel.

During the process of degradation, both the macro- and microscopic properties of the scaffold are altered. However, these alterations, as well as their potential effects on the scaffold functionality, particularly in terms of mechanical performance, are not well understood. Biodegradable polymers are degraded through four primary pathways, namely hydrolytic, oxidative, enzymatic, and physical degradation [9]. Of these, hydrolysis, which can be catalyzed by enzymes such as lipase and esterase, represents the major degradation mechanism in polymeric scaffolds [10,11]. Upon contact with water, polymer covalent bonds break, leading to smaller chains that ultimately can be eliminated from the body. Both polyesters and polycarbonates are susceptible to hydrolysis, with polyesters generally being more susceptible than polycarbonates [9].

A specific class of synthetic biomaterials are supramolecular polymers. This class is attractive for vascular TE as these materials can be customized for specific vascular applications through the incorporation of bioactive moieties, non-cell-adhesive components, or specific cell-attracting peptides [12–15]. Moreover, their properties in general are highly tunable [16,17]. For the present study we have exploited this tunability and have prepared a set of polymers with supramolecularly interacting bis-urea (BU) units in their structure. The polycarbonate-BU (PC-BU), polycarbonate-ester-BU (PC(e)-BU), and polycaprolactone-BU (PCL-BU) materials are segmented thermoplastic elastomers (TPEs) with a sequence-controlled molecular structure: the macromolecules have an exact control over hydrolysis, with polyesters generally being more susceptible than polycarbonates [9].

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2. Materials and methods

2.1. Scaffold preparation

2.1.1. Polymer synthesis

PC-BU and PCL-BU were synthesized using 3-step synthetic procedures [18,23]. Briefly, the syntheses of PC-BU and PCL-BU start from polyhexylcarbonate diol and polycaprolactone diol, respectively, with both these telechelic prepolymer diols having a M_n of 2 kDa. In the first step, the diols were capped by reaction with either 6-((tert-butoxycarbonyl)amino)hexyl 1H-imidazole-1-carboxylate applying DBU-base reagent (for PC-BU) or 6-((tert-butoxycarbonyl)amino)hexanoic acid employing DCC coupling reagent (for PCL-BU). Next, the resulting N-Boc protected telechelic polymers were deprotected with trifluoroacetic acid (TFA), and finally, in the third step, the amine functional prepolymers were converted and chain-extended with 1,4-butanediisocyanate. The synthesis of the PC(e)-BU material has also been performed in a
3-step approach, and is outlined in detail in Supplementary Information SI.1.

2.1.2. Electrospinning
Tubular scaffolds (ø 3 mm, 5 μm fibers) and scaffold sheets (10 × 10 cm², 5 μm fibers) were electrospun from three different polymer solutions containing CHCl₃ (Sigma-Aldrich, 372978), and MeOH (VWR Chemicals, 20903.368) or HFIP (Fluorochem, 920-66-1) (Table 1). The polymer solutions were delivered via a positively charged needle onto a negatively charged rotating mandrel (ø 3 mm at 500 rpm for the tubes (6 cm deposition distance) and ø 35 mm at 100 rpm for the sheets (10 cm deposition distance)) in a climate-controlled cabinet (EC-CLI, IME Technologies, Geldrop, the Netherlands) at 23 °C and 30% relative humidity. To ensure comparable fiber diameter and fiber organization between the three candidate materials, they were produced according to the settings in Table 1. After removal from the mandrel, the resulting scaffolds tubes and sheets were placed under vacuum for 16 h at 23 °C according to routinely-used protocols to remove any residual solvent [15,24]. For the experiments, 10 mm pieces were cut from the tubular meshes, and 10 × 10 mm² pieces were cut from the scaffold sheets. Prior to the degradation experiments, the samples were placed in sterile H₂O and centrifuged for 10 min at 4500 rpm to wet the materials.

2.2. Lipase accelerated degradation experiments
The prepared scaffolds were incubated in a 3 ml lipase solution in water (from Thermomyces lanuginosus, Sigma Aldrich, L0777) at concentrations ranging from 10 U/ml to 1000 U/ml at 37 °C. At each time point, scaffolds were washed 3 times with sterile H₂O, snap-frozen in liquid nitrogen, and stored at −80 °C until analysis, unless stated otherwise. Based on this protocol, three different types of degradation experiments were performed.

2.2.1. Validation experiments
In the first set of experiments, pre-wetted scaffold meshes (10 × 10 mm², n = 3 for every condition and time point) of each material were incubated at two different enzyme concentration conditions (50 U/ml and 100 U/ml lipase solution). To make direct correlations between the sample’s physical properties (i.e., scaffold thickness, mass loss, and fiber morphology, see Section 2.3.1), each analysis was performed on each individual sample at predetermined end points (0, 8, 28, 72 h).

2.2.2. Scaffold degradation and functional performance
For the second experiment, different degradation protocols were used for each material, i.e., different enzyme concentrations and/or durations were employed for each material. The 10 mm tubular PC-BU scaffolds were incubated with a 500 U/ml lipase solution up to 9 days (with intermediate time points at 3 and 6 days), whereas the PC(e)-BU and PCL-BU scaffolds were incubated with 30 U/ml and 10 U/ml lipase solution, respectively, up to 6 days (with intermediate time points at 2 and 4 days). The lipase solution was refreshed every 2–3 days to maintain enzyme activity [25,26]. During enzyme exposure, the scaffolds were monitored for their physical properties (i.e., fiber morphology, mass loss, and thickness, Section 2.3.1), chemical properties (i.e., thermal and molecular analysis, Section 2.3.2), and mechanical properties (Section 2.3.3). For each analysis, a total of three scaffolds per material per time point were included (see the detailed experimental scheme in Supplementary Information SI.2).

2.2.3. Functional performance at high scaffold degradation
In the last set of experiments, the tubular PC-BU scaffolds were incubated with a 1000 U/ml lipase solution for 20 days, while the PC(e)-BU and PCL-BU scaffolds were incubated with a 100 U/ml lipase solution for 6 and 9 days, respectively. After enzyme exposure, the sample’s mass loss (Section 2.3.1) and mechanical properties (Section 2.3.3) were characterized (n = 3 per analysis).

2.3. Experimental readouts
2.3.1. Physical properties
Fiber morphology. The scaffold fiber morphology was examined by scanning electron microscopy (SEM) (Quanta 600F, FEI, Hillsboro, OR, USA). Scaffolds were dried under vacuum, gold-sputtered, and visualized in low vacuum atmosphere with an electron beam of 5 kV. Average fiber diameters were measured from SEM images.
using ImageJ (v1.48, U.S. NIH, Bethesda, MD, USA). At least 20 individual fibers of each scaffold were measured. Fiber directionality and distribution of directionality was quantified using previously developed software [27]. The % of aligned fibers was calculated by fitting a Gaussian distribution with an additional baseline onto the histogram of the detected fiber directions. The fraction of aligned fibers was defined as the fraction of fibers belonging to the Gaussian distribution.

**Mass loss.** Scaffolds were lyophilized for 3 h, and then immediately weighed using a digital balance (XS105 dual-range analytical balance, Mettler Toledo, Switzerland). The remaining mass after scaffold degradation was normalized against the initial scaffold mass.

**Scaffold thickness.** Scaffold thickness was measured at two opposing edges (>3 locations/side) using a digital microscope (Keyence VHX-500FE, Itasca, IL, USA). For the validation experiment (Section 2.2.1 10 × 10 mm² sheets), the scaffold thickness was measured after lyophilisation, and the scaffolds dry thickness after degradation was normalized by the initial scaffold thickness. For the other experiments (Sections 2.2.2 and 2.2.3, ø3 mm × 10 mm tubes), the scaffold thickness was measured directly after washing, i.e., in wet conditions, and no normalization step was performed.

### 2.3.2. Chemical properties

**Differential scanning calorimetry analysis (DSC).** Directly after washing, scaffolds were dried under vacuum and stored at room temperature. DSC measurements were performed on a DSC Q2000 (TA instruments, USA). Electrospun samples were weighed, and subsequently hermetically sealed in Tzero aluminum pans. The samples were first cooled to −80 °C and then subjected to two heating/cooling cycles from −80 °C to 160 °C with a rate of 10 °C/min. The presented melting peak (defined as the peak maximum) and melting enthalpy (defined as the peak area) were determined from the first heating run using Universal Analysis software (V4.5A, TA instruments).

**Gel permeation chromatography (GPC).** Scaffolds samples for GPC were dissolved at a concentration of 1 mg/ml in dimethylformamide, supplemented with 10 mM LiBr and 0.3% (v/v) H₂O. Prior to the measurements, the sample solutions were filtered using a 0.2 µm regenerated cellulose filter. Weight-averaged molecular weights (Mₘ) and number-averaged molecular weights (Mₙ) relative to poly(ethylene glycol) standards were determined with a Varian/Polymer Laboratories PL-GPC 50 Plus instrument (Varian Inc., Palo Alto, CA, USA) operated at 50 °C, equipped with a Shodex GPC KD-804 column (Shodex, Tokyo, Japan).

### 2.3.3. Mechanical properties

The mechanical properties of the scaffolds were characterized immediately after washing in wet conditions at 37 °C in a biaxial tensile setup (CellScale Biomaterial Testing, Waterloo, Canada; equipped with a 1500 or 5000 nN load cell). The scaffolds were longitudinally opened and 7 × 7 mm² samples were cut. After the scaffold thickness measurement (Section 2.3.1.3), the sample’s circumferential and axial directions were aligned with the actuators and mounted. Prior to the test, the samples were sprayed with graphite to facilitate optical strain analysis. After 10 cycles of uniaxial strain up to 10% in each direction, the samples were equibiaxially stretched at a strain rate of 100% min⁻¹ until 100%. Assuming incompressibility and plane-stress conditions, Cauchy–stress–stretch curves were calculated from the force and displacement measurements. As a measure of stiffness, the slope at physiological stretch values of 1.05 and 1.15 stretch was calculated [28].

### 2.4. Statistical analysis

All data are expressed as mean ± standard deviation. To assess the overall effect of incubation time and the relationship between mass loss and scaffold thickness, the data were analyzed using linear regression in Matlab R2016b (The Mathworks, Natick, MA). To evaluate differences between the different time points, a Kruskal-Wallis test, followed by Dunn’s multiple comparison test, was performed in Prism (Graphpad Software v5.04, La Jolla, CA, USA). Statistical differences were considered to be significant for p-values <0.05.

### 3. Results

#### 3.1. Materials and scaffolds before degradation

The synthesis of the polymers resulted in biomaterials with the macromolecular structures as depicted in Fig. 2A. The sequence-controlled TPEs have identical bis-urea (BU) hard blocks, similar soft block lengths (Mₙ of about 2.5–2.8 kg/mol), but varying poly(ester/carbonate) soft block compositions. Supplementary Information SI.3 contains information on the bulk thermal and mechanical properties of the three investigated biomaterials. Electrospinning of the biomaterials resulted in fibrous scaffolds with similar fiber diameters of about 5 µm (Fig. 2B, C, Table 2). All scaffold groups exhibited at the outer side some degree of fiber alignment in the axial direction, which became more pronounced in the thicker scaffolds (Table 2, Fig. SI2A). The luminal side of the pristine scaffolds was characterized by a porous and isotropic fiber network (Fig. 2C, upper panel). Slight differences in the smoothness of fibers between the three materials were observed, which are most likely due to the electrospinning process (Fig. 2C, lower panel).

#### 3.2. Validation experiments

We first studied the degradation kinetics of the three materials, and found that the degradation profiles due to exposure of the scaffolds to 100 U/ml lipase solutions clearly differed (Fig. 3A). Similar results were obtained using 50 U/ml lipase (Supplementary Information SI.4). After 72 h incubation, the PC-BU scaffolds had hardly lost any mass (~5%), while the PC(e)-BU and the PCL-BU scaffolds had lost about 50% of their original mass (Fig. 3A). PC (e)-BU showed kinetics intermediate to those of PC-BU and PCL-BU. Only at the earliest time points it gave similar results as found for PC-BU, the material that it closely resembles molecularly. These data demonstrate that the susceptibility of the BU-based supramolecular polymeric scaffolds to enzymatic degradation can be robustly tuned.

Next, we examined and compared the fiber morphology, sample thickness, and mass loss of the scaffolds, as subjected to the two enzyme concentrations (Fig. 3B–E). For all three materials, it was observed that the varying enzyme concentrations did not affect the relationship between sample thickness and mass loss, indicating that a similar degradation state of a scaffold (i.e., a state of a scaffold of a certain mass loss coupled to a certain scaffold thickness and a certain fiber thickness, all for a particular biomaterial) can be attained using different lipase concentrations (Fig. 3C–E). This result also shows that the degradation rate does not seem to affect the degradation states that a scaffold traverses during its degradative process. Finally, a pair of scaffold samples was taken from each material group that had attained comparable degradation states after different incubation times with different enzyme concentrations (indicated by the arrowheads in Fig. 3C–E). SEM analysis of these sample pairs shows very similar fiber morpholo-
gies for each material (Fig. 3B), confirming that equivalent degradation states can be obtained by exposure to different enzyme concentrations and incubation times. These results validate our approach of examining and comparing biomaterial scaffolds as acquired by in vitro accelerated degradation protocols that apply different enzyme concentrations.

3.3. Monitoring scaffold mass and morphology upon degradation

The PC-BU, PC(e)-BU, and PCL-BU scaffolds were incubated with a 500 U/ml, 30 U/ml, and 10 U/ml lipase solution, respectively, allowing examination of the degradation states of these biomaterial scaffolds within a similar experimental time frame. The remaining mass of all scaffolds decreased during lipase exposure (Fig. 4A, see also Table SI3 in the Supplementary Information for data on statistical significance). This coincided with a decrease in the scaffold thickness for both the PC(e)-BU as well as the PCL-BU scaffolds (Fig. 4B). In contrast, the thickness of PC-BU scaffolds remained constant despite the mass loss. The scaffold density, defined as the calculated ratio between scaffold mass and thickness, decreased with longer degradation times for the PC-BU and PC(e)-BU scaffolds. In contrast, the density of the PCL-BU scaffolds largely remained constant over time, or even increased to a minor extent (Fig. 4C).

The samples were next examined at the microscopic fiber scale. Upon degradation, clear changes in the fiber morphology were observed for the three biomaterials, most strikingly for the PCL-BU scaffolds that often (but not always) showed an erosion-like...
appearance with small pits in the fibers (Fig. 5A). Quantification of the fiber diameters from SEM images revealed a constant, even slight increase of fiber diameter in the PC-BU scaffolds, although this was not statistically significant (Fig. 5B). In contrast, the fiber diameters in PC(e)-BU and PCL-BU scaffolds gradually and significantly decreased with longer enzymatic degradation.

To assess the homogeneity of fiber degradation in the samples, the fiber diameter as a function of mass loss was plotted (Fig. 5C). If the material degrades homogeneously via surface erosion, the relation between the decrease in fiber diameter and mass loss is expected to follow a quadratic profile (dotted lines in Fig. 5C, see Supplementary Information SI.6). The PCL-BU and PC(e)-BU fibers degrade corresponding to this profile, although this is less clear for the PC(e)-BU material. On the other hand, the profile for the PC-BU fibers seems to show a slight positive correlation with mass loss, suggesting fiber swelling.

3.4. Monitoring thermal properties and molecular weight upon degradation

In further analyses, we checked whether enzymatic degradation resulted in changes in the thermal properties and the molecular weight of the remaining scaffold materials. The trace of the first heating run from the DSC measurements on the electrospun scaffolds typically showed two distinct transitions (Fig. 6A). The first melting transition corresponds to the melting of the polymer soft phase (i.e., PC, PC(e) or PCL), whereas the second transition (peak at >100 °C) corresponds to the melting of the BU hard phase. Only for PCL-BU an additional third transition was observed at about 60 °C (see Supplementary Information SI.3 for related details). The melting enthalpy as well as the melting temperature of the BU-melt did not significantly change for the three polymers upon degradation, even though they tended to decrease with enzymatic degradation, especially for the PCL-BU polymer (Fig. 6B, C).

Finally, the GPC measurements indicated that the molecular weight of the remaining PC-BU and PC(e)-BU materials did not change with degradation. For PCL-BU, however, the molecular weight of the remaining material slightly and significantly decreased (Fig. 6D). Apparently, the bulk material in the fibers is not affected by lipase and/or water for PC-BU and PC(e)-BU, while it degrades for PCL-BU.

3.5. Monitoring scaffold mechanical properties upon degradation

To assess the mechanical performance of the scaffolds after degradation, biaxial tensile testing was performed. All scaffolds showed some degree of non-linear, anisotropic mechanical behavior (Fig. 7A). In particular, the PC-BU scaffolds were stiffer in the circumferential direction at higher levels of stretch, whereas the PC(e)-BU and PCL-BU scaffolds were stiffer in the axial direction. Irrespective of the polymer backbone, the pristine materials showed E-moduli in the same range of about 1 MPa, confirming...
the similarity between the mechanical properties of the three biomaterials. Interestingly, the PCL-BU scaffolds became stiffer upon degradation in the axial direction, even at a high degree of degradation (Fig. 7B, Table 3). This stiffening effect was also observed, though only at higher stretches, for PCL-BU scaffolds that were deliberately electrospun to acquire fibers in the circumferential direction (see Supplementary Information SI.7 for details). The PC(e)-BU scaffolds largely maintained their mechanical performance up to 40% of remaining mass (Fig. 7B). However, these scaffolds abruptly lost their tensile properties at higher levels of degradation (Table 3). The behavior of the PC-BU scaffolds was less clear, but it seemed that their E-moduli stayed in the same range upon degradation (Fig. 7B, Table 3).

4. Discussion

In in situ TE, the degradation rate of the porous implant should complement the rate at which new tissue is formed to warrant a sustained mechanical function. Patient characteristics (e.g., immune response and age), location of implantation [21,22], and the specific nature of the scaffold (e.g., shape, chemical composition, physical properties) determine the degradation rate of the implant. Ultimately, the in situ TE process is aimed at, and must lead to, a safe and successful transition from synthetic conduits into neo-vessels in which no scaffold is present anymore. Importantly, during this transition, the mechanical integrity of the implant must be maintained at all times to prevent premature graft failure. Here, we therefore have dissected how enzymatic hydrolytic degradation affects the physical properties and mechanical performance of scaffolds as electrospun from the candidate supramolecular BU-materials PC-BU, PC(e)-BU, and PCL-BU. We have demonstrated that the electrospun scaffolds degrade in different ways, and based on the findings we propose a material dependent degradation mechanism for the scaffolds that is schematically visualized in Fig. 8.

The examined BU-materials have a sequence-controlled molecular structure, and in this respect they deviate from polycarbonate, polyester, or co-polycarbonate/ester BU-materials that are prepared in two-step one-pot procedures [29,30]. The latter TPE materials have a macromolecular structure that is determined by statistics, and that accordingly has a range in hard and soft block identities, implying that all macromolecules composing a certain material have a different molecular microstructure. These materials also contain urethane groups, while PC-BU, PC(e)-BU, and PCL-BU do not. Each of the three sequence-controlled biomaterials is composed of macromolecules that are very much alike, and that only vary in macromolecular length. Accordingly, the degradation products of PC-BU, PC(e)-BU, and PCL-BU can be expected to be less diverse than those for the one-pot produced BU-polyurethanes. The high control over their molecular structure, leading to a high control over their specific properties, make sequence-controlled materials attractive for assessing their performance in biomedical applications, for example in in situ TE.
PC-BU, PC(e)-BU, and PCL-BU are soluble in a range of solvents and can therefore be developed for solvent processing by electrospinning, resulting in fibrous scaffolds with non-linear and anisotropic mechanical properties. Scaffolds can be produced with about 5 μm fibers and open porous structures that allow infiltration of cells. One-pot produced BU-polyurethanes are less soluble, but can nevertheless be electrospun from HFIP to acquire scaffold meshes with (sub)micrometer fibers and relatively dense structures [31,32]. The PCL, PCL/PC and PC-BU-polyurethanes have also been examined in in vitro degradation assessments (on solid films exposed to PBS), and in in vivo degradation studies (on salt-leached scaffolds subcutaneously implanted in rats), and the results show a gradient in degradative behavior for the explored materials [29]. Although these investigations are related to this work, the experiments were not performed on electrospun scaffolds, precluding further direct comparisons.

In line with previous reported results on PCL-BU [26], PCL-BU scaffolds became more stiff with degradation (Fig. 7B, Table 3). We reasoned that the changes in mechanical properties were the result of changes at the network level (i.e., physical properties of fibers and scaffold) and at the material level (i.e., thermal properties and molecular weight). Indeed, the fibers in the PCL-BU scaffolds displayed surface erosion, as was monitored up to a substantial mass loss for the scaffold (Fig. 5C). At the material level, the hard block melting enthalpy and melting temperature (Fig. 6B, C) as well as the molecular weight (Fig. 6D) of PCL-BU tended to decrease with mass loss of the scaffold, indicating a deterioration in crystallinity [33] and, importantly, bulk degradation. This assessment is corroborated by SEM, showing affected rough-surfaced fibers with dents and pits (Fig. 5A), and with data from a comparable study that also showed a slight decrease in molecular weight of PCL-BU scaffolds after 30% mass loss as a result of lipase exposure [26]. Finally, the studied PCL-BU scaffolds showed a minor increase in the macroscopic density upon degradation (Fig. 4C), indicating a collapse of the fibrous network. Taken together, enzymatic hydrolytic degradation affected the polyester PCL-BU fibers from the outside and from the inside, and this presumably promoted the network to collapse, which in turn led to an overall stiffening of the scaffold.

It is important to notice that all scaffolds displayed anisotropic mechanical behavior at all time points. This anisotropic behavior is attributable to the higher degree of fiber alignment in the axial direction, especially for the PC(e)-BU and PCL-BU scaffolds, as a result of the electrospinning process (Table 2, Fig. SI2A). Interestingly, the stiffening effect in the PCL-BU scaffolds seemed to occur in the direction of the main fiber orientation (i.e., axial stiffening in axially aligned scaffolds and circumferential stiffening in circumferentially aligned scaffolds, Supplementary Information SI.7). The apparent relation between degradation and structural anisotropy adds an extra complexity to the design of scaffolds for in situ TE, which should be appropriately addressed with for example constitutive modeling [34]. This also holds for the non-linear mechanical behavior that these electrospun scaffolds exhibit. Due to the combined non-linearity and anisotropy, loading configurations are likely to vary during degradation, thereby influencing the regeneration process. The change in loading configuration during scaffold degradation could be captured with constitutive models as well, and used to optimize scaffold design.

The PC-BU scaffolds, on the other hand, maintained their mechanical properties during enzyme-accelerated hydrolytic degradation, although the mechanical properties were more influenced by the solvent processing method than by the material composition. PC-BU scaffolds are less soluble than the other two materials, which makes them less susceptible to degradation by electrospinning. This suggests that solvent processing methods may play a crucial role in determining the mechanical properties of electrospun scaffolds, and that careful consideration should be given to the selection of solvent and processing conditions.
in vitro degradation (Fig. 7B, Table 3). This result is in line with previous work, albeit that degradation was only followed up to 5% mass loss [18]. At the network level, PC-BU fiber diameters increased (Fig. 5C) and scaffolds remained of constant thickness (Fig. 4B), despite the mass loss, suggesting that surface erosion in the PC-BU scaffolds is accompanied by swelling of the fibers with water. Swelling by absorption of water is known for PCL-based BU-polyurethanes, so it is not uncommon for BU-materials [35]. Chemical characterization of the PC-BU scaffolds indicated that the enzymes did not affect the material remaining in the fibers (Fig. 6), confirming that PC-BU solely degrades by surface erosion and not by bulk degradation. Consequently, the fibrous network gave stable mechanical moduli upon degradation up to about a 40% mass loss.

Finally, we introduced a new synthetic biomaterial, PC(e)-BU; a polymer that very closely resembles PC-BU with respect to its macromolecular structure. Surprisingly, however, PC(e)-BU scaffolds degrade at a rate that is more comparable to that of PCL-BU scaffolds, although PC(e)-BU initially deteriorates slower than PCL-BU (Fig. 3A). The ester bonds in PC(e)-BU are in close proximity to the stacking and crystallizing bis-urea (BU) groups, but apparently they are still quite accessible for cleavage by the lipase enzyme. At the material level, PC(e)-BU did not show signs for bulk degradation (Fig. 6D), which is similar to PC-BU. At the network level, the PC(e)-BU scaffolds were also more resembling the PC-BU scaffolds, as the fibers seemed to show surface erosion without signs of a collapsing network despite the significant reduction in fiber diameters (Figs. 4C and 5B, C). However, at extreme levels of degradation (ca. 80% mass loss), the PC(e)-BU scaffolds abruptly lose their mechanical properties, while remarkably the PCL-BU scaffolds retain their modulus (Table 3). In this respect, the performance of PC(e)-BU is inferior to that of PCL-BU, as for the latter the mechanical robustness is warranted over a broader degradation range.

Degradation of scaffolds in vivo is highly complex, involving the interplay between scaffold (e.g., fiber diameter, fiber alignment, pore size, and substrate stiffness), cells (e.g., macrophages and tissue producing cells [36–38]), and hemodynamics (e.g., shear stress and cyclic strain [28]). Our study highlights that through a simplification of these complex environments and a thorough examination of the physical, chemical, and mechanical changes during enzymatic degradation, it is possible to identify the degradation mechanisms of scaffolds that are designed for in situ TE applications. We therefore expect that this highly-controlled in vitro test...
ing approach can be useful to also reveal the degradation mechanisms of materials in general that relate to, for example, oxidation and physical loads [26].

5. Conclusions and outlook

We have shown that electrospun vascular scaffolds made from a set of sequence-controlled BU-modified biomaterials with varying amounts of ester groups in the polymer backbone are degraded through different mechanisms when exposed to lipase. PC-BU and PC(e)-BU polycarbonate scaffolds, with respectively a low and intermediate susceptibility to degradation, degrade through surface erosion, resulting in maintenance of the scaffold’s mechanical properties. PCL-BU polyester scaffolds, with a high susceptibility to degradation, degrade through surface erosion and some bulk degradation, which ultimately is accompanied by a network collapse, resulting in overall stiffening of the scaffold. Overall, it is observed that enzymatic hydrolytic degradation of electrospun scaffolds can be slowed down effectively when PC-BU is used, a material with only bis-urea (BU) and carbonate groups and without ester or urethane moieties. These results aid in the selection of electrospun biodegradable polymeric scaffolds for in situ TE, for instance for the preparation of small-diameter vascular substitutes [6,7,39].

The introduction of PC(e)-BU to the set of BU-modified supramolecular polymers opens new opportunities to further tune the degradation kinetics of scaffold implants. In contrast to polymers in general, PC(e)-BU and PC-BU can, due to their macromolecular similarity, be combined to acquire an intimately mixed polymer blend (Supplementary Information SI.3). Since their degradation mechanisms are alike, but their degradation rates vary, mixtures of these polymers are expected to resorb at inter-
mediate rates. This mix-and-match approach illustrates that the class of supramolecular polymers continues to represent a promising group of materials for use in (vascular) TE approaches in particular, and biomedical applications in general.

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Author contributions

E.E.v.H., R.D., N.A.K., P.Y.W.D., and C.V.C.B. conceptualized the study. S.H.M.S., M.H.C.J., and H.M.J. designed, developed and synthesized the polymers. E.E.v.H. and R.D. performed the experiments and analyzed the data. B.D.I. performed the chemical analyses. E.E.v.H. and R.D. wrote the article. All authors were involved in the review and editing of the article. N.A.K., A.I.P.M., P.Y.W.D., and C.V.C.B. supervised the project. P.Y.W.D. and C.V.C.B. managed the research and acquired funding to execute the project.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.actbio.2019.05.037.

References


Fig. 8. Schematic illustrating the proposed degradation mechanisms for scaffolds of the three tested sequence-controlled BU-materials with varying susceptibilities to enzymatic degradation.