

# Effect of biomimetic conditions on mechanical and structural integrity of PGA/P4HB and electrospun PCL scaffolds

Leda Klouda · Claudia M. Vaz · Anita Mol ·  
Frank P. T. Baaijens · Carlijn V. C. Bouten

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**Abstract** The selection of an appropriate scaffold represents one major key to success in tissue engineering. In cardiovascular applications, where a load-bearing structure is required, scaffolds need to demonstrate sufficient mechanical properties and importantly, reliable retention of these properties during the developmental phase of the tissue engineered construct. The effect of in vitro culture conditions, time and mechanical loading on the retention of mechanical properties of two scaffold types was investigated. First candidate tested was a poly-glycolic acid non-woven fiber mesh, coated with poly-4-hydroxybutyrate (PGA/P4HB), the standard scaffold used successfully in cardiovascular tissue engineering applications. As an alternative, an electrospun poly- $\epsilon$ -caprolactone (PCL) scaffold was used. A 15-day dynamic loading protocol was applied to the scaffolds. Additionally, control scaffolds were incubated statically. All studies were performed in a simulated physiological environment (phosphate-buffered saline solution,  $T = 37^\circ\text{C}$ ). PGA/P4HB scaffolds showed a dramatic decrease in mechanical properties as a function of incubation time and straining. Mechanical loading had a significant effect on PCL scaffold properties. Degradation as well as fiber fatigue caused by loading promote loss of mechanical properties in PGA/P4HB scaffolds. For PCL,

fiber reorganization due to straining seems to be the main reason behind the brittle behavior that was pronounced in these scaffolds. It is suggested that those changes in scaffolds' mechanical properties must be considered at the application of in vitro tissue engineering protocols and should ideally be taken over by tissue formation to maintain mechanically stable tissue constructs.

## Introduction

In order to be used in cardiovascular tissue engineering applications, a scaffold has to fulfill certain requirements in terms of mechanical properties. Cardiovascular tissues serve a predominantly biomechanical function and the scaffold should maintain a physical support until the extracellular matrix secreted by the cells is capable of carrying the loads to which the tissue engineered construct is subjected. It has been proven that dynamic in vitro stimulation enhances tissue formation and bioreactors have been developed for that purpose [1–4]. Typically, desired scaffold mechanical properties involve relevant initial tensile strength, modulus and elastic behavior, but also sufficient retention of these properties during in vitro or in vivo development of the tissue engineered construct. The maintenance of scaffold properties is of paramount importance especially during the early stages of in vitro conditioning while the tissue is growing and does not yet possess adequate mechanical integrity. Additionally, the scaffold should maintain elasticity without significant permanent deformation in order to transduce the appropriate mechanical stimuli to the cells.

The characterization of scaffolds' mechanical behavior is often performed in dry state under ambient conditions or in air at  $37^\circ\text{C}$  [5–11]. Thompson et al. [12], as well as

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L. Klouda · C. M. Vaz · A. Mol · F. P. T. Baaijens ·  
C. V. C. Bouten  
Division of Biomechanics and Tissue Engineering, Department  
of Biomedical Engineering, Eindhoven University of  
Technology, P.O. Box 513, Eindhoven 5600 MB,  
The Netherlands

L. Klouda (✉)  
Department of Bioengineering, Rice University, MS-142,  
P.O.Box 1892, Houston, TX 77251-1892, USA  
e-mail: leda.kloudas@rice.edu

other researchers [4, 13–16] have addressed the importance of determining mechanical properties under biomimetic conditions, including hemodynamic forces that represent the physiological and mechanical environment in which the scaffolds are actually required to perform.

The aim of this work is a systematic approach to investigate the effects of *in vitro* culture (aqueous environment, pH = 7.4, T = 37 °C) and mechanical conditioning on the retention of mechanical properties over time. Two different biodegradable synthetic polymer scaffold types were tested: a non-woven poly-glycolic acid mesh coated with poly-4-hydroxybutyrate (PGA/P4HB) and an electrospun poly- $\epsilon$ -caprolactone (PCL) mesh. PGA/P4HB is a biomaterial that has been used extensively with promising results for cardiovascular tissue engineering applications such as heart valves [3, 17] and vascular grafts [18]. It has resulted in the first successful demonstration of a tissue engineered tri-leaflet heart valve in a sheep model [17]. The addition of P4HB mainly contributes to the scaffold's pliability and strength [17, 19] through fiber entanglement. This scaffold exhibits a relatively quick degradation profile, with complete bioabsorption *in vivo* after 4 (PGA) and 8 (P4HB) weeks [17]. Electrospun PCL scaffolds are currently evaluated in our group for their potential in heart valve tissue engineering [20]. PCL shows a highly elastic behavior combined with a longer degradation time [20–22]. Electrospinning of the material provides a mesh with high porosity and small diameter fibers, two features that are desirable for tissue engineering applications. Additionally, this technique allows easy molding of scaffolds in any desired shape e.g. that of a heart valve [20].

In this study, the mechanical properties, morphology, degradation and thermal behavior of both scaffold types at pure state have been determined and compared to those of samples after a 2-day static, after a 15-day static as well as after a 15-day dynamic incubation period under *in vitro* culture conditions (aqueous environment, pH = 7.4, T = 37 °C). Dynamic tension, provided by a custom-made bioreactor, has been selected as a stimulus to scaffolds as it represents an important type of mechanical loading in cardiovascular and other tissues and has been shown to enhance the development and function of tissue engineered blood vessels [14], heart valve [3] and smooth muscle [23, 24] tissues.

## Materials and methods

### Scaffolds

A non-woven poly-glycolic acid mesh (PGA; molecular weight: company confidential information, thickness: 1.0 mm, specific gravity: 71 mg/cm<sup>3</sup>, melting point:

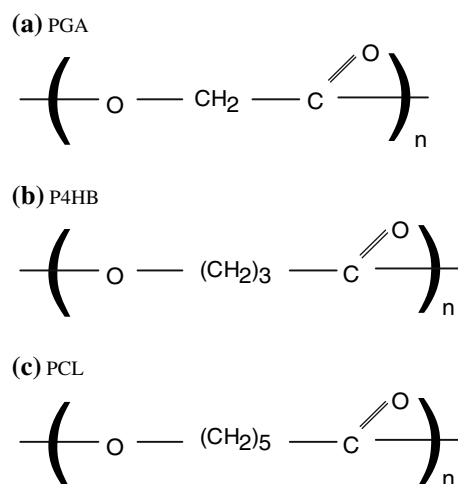
225 °C, approximate fiber diameter: 12–15  $\mu$ m, Cellon S.A., Luxembourg) (Fig. 1a) was coated with a thin layer of P4HB (molecular weight: 1,000 kDa, melting point: 60 °C, Tephra Inc., MA, USA) (Fig. 1b) by dipping briefly in a 1% (w/v) P4HB solution in tetrahydrofuran and vacuum dried for solvent evaporation.

A non-woven PCL mesh was produced by electrospinning as previously described [20] (Fig. 1c). In brief, a 12.5% (w/v) PCL (average molecular weight 80 kDa, melting point 57 °C, Sigma-Aldrich, The Netherlands) solution in chloroform (Sigma-Aldrich, The Netherlands) was delivered through a capillary under high voltage and ejected, assisted by electrostatic forces, resulting in a non-woven fibrous mesh and solvent evaporation. The PCL meshes have a thickness of 0.3 mm, a specific gravity of 210 mg/cm<sup>3</sup> and fiber diameters ranging between 0.6 and 3.0  $\mu$ m.

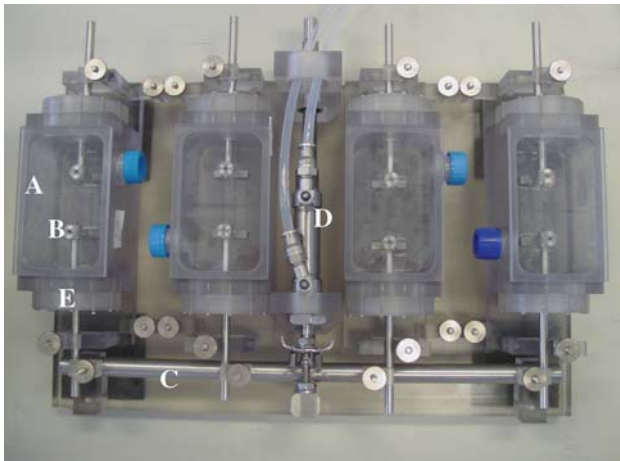
All scaffolds were cut in rectangular pieces with dimensions of 35 × 8 mm.

### Bioreactor

A custom-made bioreactor (Fig. 2) was used to apply cyclic strain to the scaffolds. The bioreactor consists of four identical vented culture chambers (A) with two stainless steel clamps (B) to attach samples. One clamp per chamber is coupled to a rigid arm (C), which is connected to a pneumatic drive (D) (Festo, Germany). The amplitude of the pneumatic drive movement can be adjusted and is expressed as percentage tensile strain of the initial clamped sample length. Silicone membranes maintain sterility (E). Each bioreactor chamber was filled with phosphate-buffered saline solution (PBS; pH = 7.4, Sigma, Germany), so that scaffolds were completely immersed. The whole set-up



**Fig. 1** Chemical structures of: (a) poly-glycolic acid (PGA), (b) poly-4-hydroxybutyrate (P4HB), and (c) poly- $\epsilon$ -caprolactone (PCL)



**Fig. 2** Bioreactor used for the application of dynamic strains on scaffolds. In each of the four identical chambers (A) samples can be attached between two stainless steel clamps (B). One clamp per chamber is coupled to a rigid arm (C), which is connected to a pneumatic drive (D). The amplitude of the pneumatic drive movement can be adjusted and is expressed as percentage tensile strain of the initial clamped sample length. Silicone membranes maintain sterility (E). The bioreactor can be operated inside a standard cell culture incubator

was placed inside a humidified incubator (37 °C, 5% CO<sub>2</sub>) during the complete test period.

#### Dynamic and static incubation experiment

Samples ( $n = 8$  per scaffold type) were mechanically conditioned for a total period of 15 days. During the first week gradually increasing strains from 2 to 10% were applied by introducing 2% strain for the first 36 h, then 4% for the following 36 h and continuing with a gradual increase by 2% until reaching 10% strain after 1 week. For the remaining test period, a constant dynamic strain of 10% was applied. The selected strain magnitude, protocol and frequency (1 Hz) have been proven to enhance tissue development in tissue engineered heart valve constructs over a 15-day period [3]. Two separate runs were conducted for PGA/P4HB and PCL accordingly.

The permanent deformation of the PGA/P4HB scaffolds during mechanical conditioning was corrected for, using the equation:  $y = 0.25x^2 - 0.14x - 0.02$ , where  $x$  represents the percentage applied strain and  $y$  represents the deformation as a percentage of the applied strain, as previously determined by Mol et al. [3]. The deformation expected at every strain magnitude was calculated from this equation. The straining protocol was adjusted at every strain increase, by adding to the elongation that would produce the initial straining level the expected deformation, resulting in the desired strain magnitude.

PCL scaffolds show an elastic behavior with minor permanent deformation for strains up to 10% in dry state [20], and therefore no correction was deemed necessary. The initial length of the scaffolds was recorded as well as the final length after straining for determining any permanent deformation.

Corresponding static scaffold samples were maintained unloaded in culture wells filled with PBS inside the incubator for up to both 2 ( $n = 6$  per scaffold type) and 15 ( $n = 6$ ) days.

Samples of pure dry scaffold material ( $n = 6$  per scaffold type) were used as reference.

#### Tensile testing

A custom-made uniaxial tensile testing machine with a 20-N load cell was used to obtain engineering stress-strain curves. Tensile tests were performed at room temperature on wet material (in case of strained and static scaffolds) and dry material (in case of pure scaffolds). The cross-head speed was 0.1 mm/s and the gauge length 15 mm. Conditioned samples were tested in the direction of the applied strain. Scaffolds were preconditioned with three cycles of 10% strain and then pulled to break to determine ultimate tensile strength (UTS) and elongation at failure. The incremental Young's modulus (E) was calculated from the slope of the stress-strain curve at its initial linear section at the strain interval of 5–10%. Information on the elastic behavior was obtained from all loading cycles.

#### Degradation studies

After tensile testing, scaffolds were rinsed twice with distilled water and vacuum-dried at 37 °C. Polymer mass loss was obtained by comparing the dry mass of the samples after the experiment to their original dry mass, which had been determined after vacuum-drying.

#### Differential scanning calorimetry (DSC)

Analysis was performed in a Differential Scanning Calorimeter (Q100, TA Instruments, USA). Temperature was increased at a rate of 10 °C/min over the range of –100 °C to 80 °C for PCL scaffolds and from –100 to 250 °C for PGA/P4HB scaffolds. The degree of crystallinity for PCL scaffolds was calculated from the melting peak of the polymers using the enthalpy of fusion for 100% crystalline PCL (135 J/g, [25]) as a standard. Due to experimental difficulties in obtaining the weight fractions of PGA and P4HB in the composite scaffolds in order to calculate their crystalline fractions, these crystallinities are not reported.

## Environmental scanning electron microscopy (E-SEM)

Scanning electron microscopy was performed on dynamically conditioned, static and pure samples. Vacuum-dried samples were attached to aluminum stubs and sputter-coated with a thin layer of gold (thickness < 50 nm) using a Fine Coat Ion Sputter (JFC-1100, JEOL Inc, USA). Micrographs were obtained by an environmental scanning electron microscope (E-SEM, Philips XL30 Esem-FEG, Eindhoven, The Netherlands) using a secondary electrons (SE) mode under high vacuum ( $10^{-5}$ – $10^{-6}$  mbar) and an accelerating voltage of 1 kV (PCL scaffolds) and 2 kV (PGA/P4HB scaffolds). The average fiber diameter was obtained from E-SEM micrographs. About 15 fibers were measured per sample.

## Statistical analysis

Data are expressed as mean  $\pm$  standard error. Results were statistically analyzed using the Statgraphics Plus 5.1 software package. Test groups were compared using the Kruskal-Wallis test and differences were considered statistically significant when  $p < 0.05$ .

## Results

### Overall scaffold behavior after dynamic incubation experiment

After 15 days of straining in the bioreactor, PGA/P4HB scaffolds were intact but showed a permanent deformation of  $13.0 \pm 0.5\%$  in relation to the original length.

Intact PCL scaffolds ( $n = 4$  out of  $n = 8$ ) exhibited a permanent deformation of  $4.0 \pm 0.3\%$ .

### Tensile properties

#### PGA/P4HB

Incremental Young's modulus of the pure scaffold samples was  $1.36 \pm 0.26$  MPa. Values were significantly lower for samples incubated under both static and dynamic conditions ( $p = 0.0004$ ). After 2-day static, 15-day static and 15-day dynamic incubation,  $E$  was  $0.45 \pm 0.06$  MPa,  $0.24 \pm 0.04$  MPa and  $0.46 \pm 0.04$  MPa respectively. However, differences between the latter three groups were not statistically significant.

UTS of the pure scaffold samples was  $0.22 \pm 0.03$  MPa. No significant difference was observed after a 2-day static incubation ( $0.16 \pm 0.01$  MPa). After 15-day static and dynamic incubation values decreased significantly ( $0.03 \pm 0.01$  MPa and  $0.04 \pm 0.01$  MPa respectively,

$p = 0.0004$ ), suggesting that UTS is not maintained over time (Fig. 3).

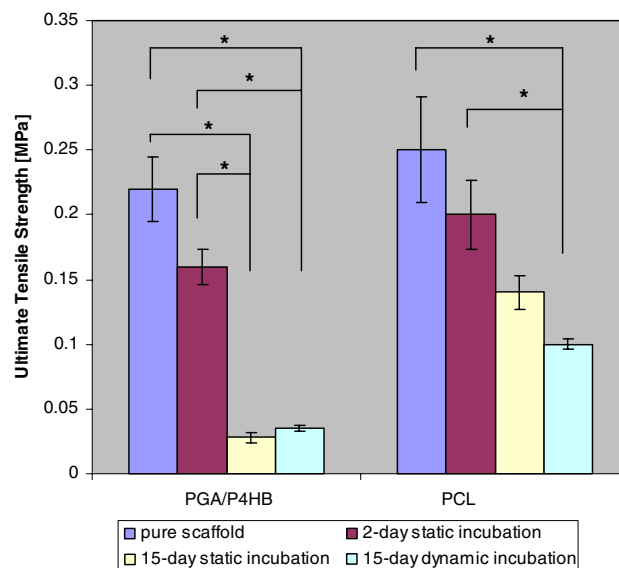
Elongation at failure of pure scaffold samples was  $45 \pm 1\%$ . After 2-day static incubation values did not show any significant change ( $48 \pm 2\%$ ). Values decreased significantly ( $p = 0.0002$ ) at 15-day incubation, both under static ( $29 \pm 1\%$ ) and dynamic ( $15 \pm 1\%$ ) conditions (Fig. 4).

It was not possible to obtain any information about the elastic range from stress–strain curves of PGA/P4HB scaffold samples in any of all four experimental groups. Additionally, a hysteresis was observed between every loading cycle.

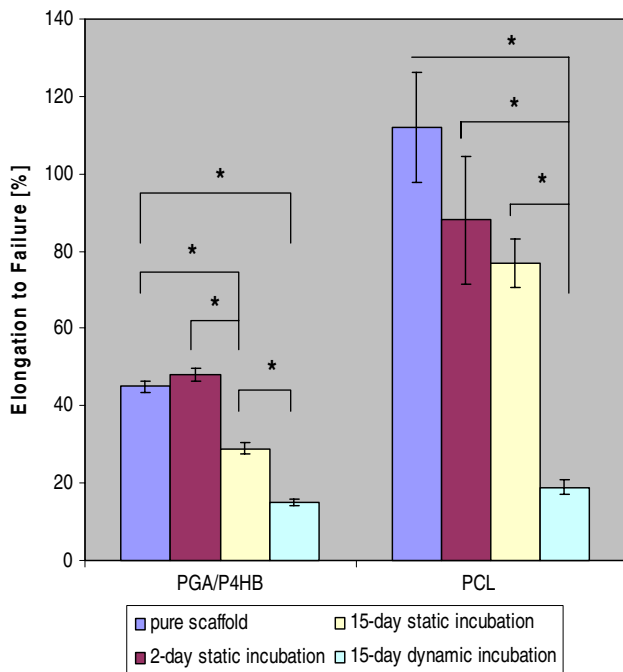
#### PCL

Incremental Young's modulus of the pure scaffolds was  $1.59 \pm 0.33$  MPa. After 2-day static, 15-day static and dynamic incubation, values decreased to  $1.18 \pm 0.04$  MPa,  $1.11 \pm 0.07$  MPa and  $1.13 \pm 0.05$  MPa respectively. Differences between groups were not statistically significant ( $p = 0.18$ ).

UTS of pure scaffold samples was  $0.25 \pm 0.04$  MPa. Values decreased for samples incubated under both static and dynamic conditions. After 2-day static incubation, UTS was  $0.20 \pm 0.02$  MPa. After 15-day incubation, values were  $0.14 \pm 0.01$  MPa (statically) and  $0.10 \pm 0.01$  MPa (dynamically). The decrease of the latter was statistically significant in comparison to pure and 2-day static samples with  $p = 0.0003$  (Fig. 3).



**Fig. 3** Ultimate tensile strength of PGA/P4HB and PCL scaffolds in pure state, after 2-day static, 15-day static and 15-day dynamic incubation in PBS at 37 °C. Bars indicate standard error. Statistically significant differences are marked with an asterisk



**Fig. 4** Elongation at break of PGA/P4HB and PCL scaffolds in pure state, after 2-day static, 15-day static and 15-day dynamic incubation in PBS at 37 °C. Bars indicate standard error. Statistically significant differences are marked with an asterisk

Elongation at failure of pure scaffold samples was  $112 \pm 14\%$ . Also here values decreased with time for samples incubated both under static as well as dynamic conditions. After 2-day and 15-day static incubation values of elongation at break of  $88 \pm 16\%$  and  $77 \pm 6\%$  were observed respectively. A significant decrease ( $p = 0.0003$ ) was observed for 15-day strained scaffold samples, which showed an elongation at break of  $19 \pm 2\%$  (Fig. 4).

Elasticity was maintained up to 10% strain in all four experimental groups as determined by stress–strain curves. However, a deformation in the range of 2% could be observed in pure and 2-day strained PCL samples between the first and second loading cycle. In 15-day samples, a deformation of 3–4% was observed. This hysteresis phenomenon might be explained due to fiber reorientation, as previously reported [20].

### Degradation studies

A mass loss of 0.9% was observed at PGA/P4HB scaffold samples at 2-day static incubation. At 15-day static incubation time, the mass loss increased to 11.3%. Dynamic straining further accelerated mass loss which increased to 18.0% at day 15 (Fig. 5).

No mass loss was found for PCL even after 15-day static and dynamic incubation.

### Differential scanning calorimetry

The thermal behavior of the four experimental groups was examined by DSC. Typical DSC thermograms for every scaffold are depicted in Figs. 6 and 7.

In pure PGA/P4HB scaffolds, PGA showed a peak at the melting temperature ( $T_m$ ) of approximately 220 °C and P4HB at 58 °C. A shift towards lower melting temperatures was observed on PGA after 15 days, both under static and dynamic conditioning. No similar shift was observed on P4HB in any of the experimental groups (Fig. 6).

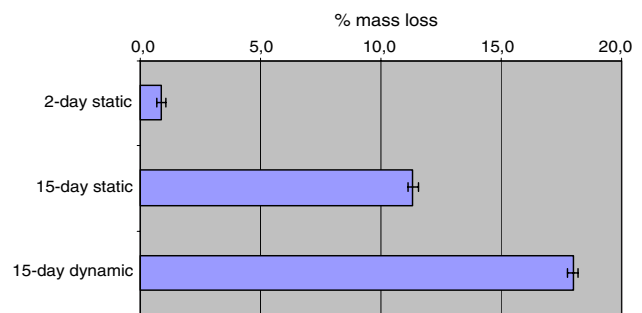
Pure PCL scaffolds demonstrated a  $T_m$  of approximately 54 °C. This melting temperature did not seem to change for any of the experimental groups (Fig. 7). Changes in crystallinity for PCL were revealed (Table 1). Crystallinity showed an increasing trend with time and dynamic conditioning; however the differences were not statistically significant.

### Environmental scanning electron microscopy

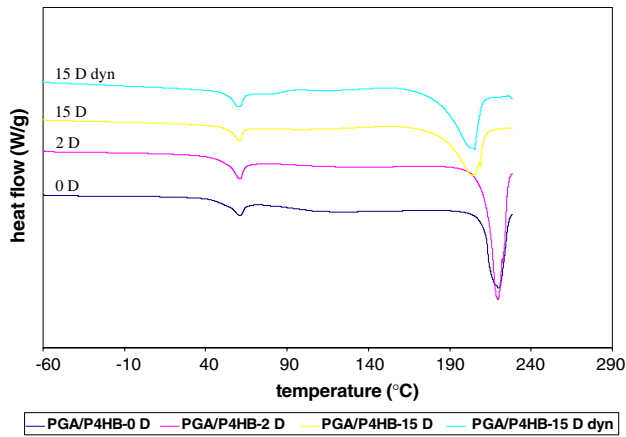
Several observations could be made from scanning electron micrographs.

#### PGA/P4HB

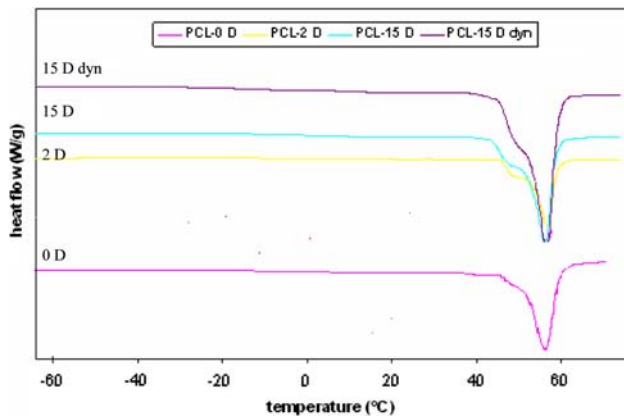
On pure scaffold samples, fibers appear to be of consistent diameter and with random orientation (Fig. 8a). The P4HB coating does not appear to be uniformly distributed over the fiber structure but is rather localized, providing some entanglement points. Fiber rupture is demonstrated on 15-day samples, with more pronounced fragmentation in strained scaffolds (Fig. 8b and 8c). Further, on 15-day strained samples a fiber reorientation seems to have occurred towards strain direction. Entanglement points provided by P4HB coating, especially in strain direction, are also partially ruptured (Fig. 8c). On pure scaffolds, the average fiber diameter was  $20.7 \pm 0.9 \mu\text{m}$  as derived from



**Fig. 5** Mass loss for PGA/P4HB scaffolds after 2-day static, 15-day static and 15-day dynamic incubation in PBS at 37 °C. Bars indicate standard error. All groups show statistically significant differences



**Fig. 6** DSC curves of PGA/P4HB scaffolds in pure state, after 2-day static, 15-day static and 15-day dynamic incubation in PBS at 37 °C. The first peak on every curve corresponds to the melting point of P4HB and the second to the melting point of PGA

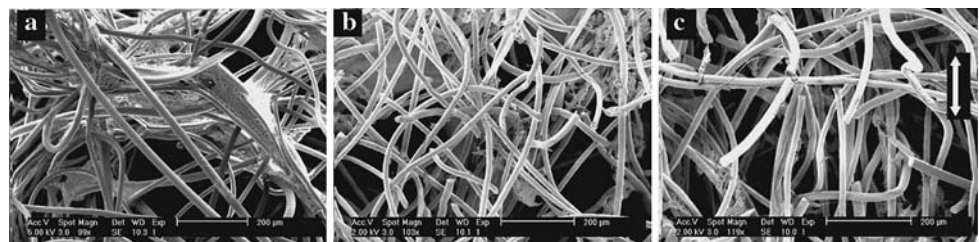


**Fig. 7** DSC curves of PCL scaffolds in pure state, after 2-day static, 15-day static and 15-day dynamic incubation in PBS at 37 °C. The peaks correspond to the melting point of PCL

**Table 1** Fraction of crystalline part in PCL scaffolds (%)

% Crystallinity				
Pure scaffold	2-day static	15-day static	15-day dynamic	
39.96 ± 0.16	44.85 ± 0.83	46.55 ± 1.99	50.11 ± 2.22	

**Fig. 8** Representative E-SEM micrographs from PGA/P4HB scaffolds in pure state (a), after 15-day static (b) and 15-day dynamic incubation in PBS at 37 °C (c). Strain direction is indicated with an arrow on 5c. Micrographs are depicted in 100-fold magnification



fiber measurements on E-SEM micrographs. Although there is a trend towards average fiber diameter decrease with time of incubation and straining, results did not prove to be statistically significant.

### PCL

In the pure scaffold as well as in all other groups, mesh is inhomogeneous and a broad fiber diameter distribution is present. Fiber structure and orientation appears to be random (Fig. 9a). The micrographs obtained from scaffolds from all experimental groups revealed a big variation in fiber distribution and architecture within and across samples and therefore no comparisons can be made (Fig. 9b).

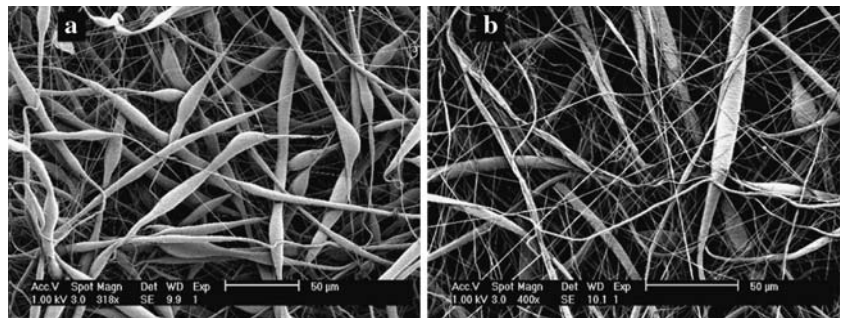
### Discussion

Researchers often determine the mechanical properties of their scaffolds under ambient conditions or in air at 37 °C [5]. In accordance with [4, 12–16], we propose that mechanical properties should be tested under the conditions that simulate the actual “work environment” of the scaffolds, either in vitro or in vivo. Therefore, in the present study a systematic approach to evaluate the effects of the in vitro conditioning parameters on the mechanical properties of cardiovascular tissue engineering scaffolds has been made. In addition, structural and morphological changes of the scaffolds have been correlated to any altered mechanical behavior.

Two different scaffold types as candidates for cardiovascular tissue engineering applications were used in this study: a non-woven PGA/P4HB mesh, the most successfully used up to now in this area, and an electrospun PCL mesh, a scaffold that is currently being evaluated in our group.

We investigated the effect of the in vitro culture environment (aqueous solution, pH = 7.4, T = 37 °C) by incubating scaffolds under static conditions for a period of 2 days. Moreover, incubating scaffolds under static conditions for 15 days tested the effect of incubation time on their properties. Dynamic straining, as a form of cyclic mechanical loading implemented during in vitro culture of tissue engineered constructs, was applied as an additional

**Fig. 9** Representative E-SEM micrographs from PCL scaffolds in pure state (a) and after 15-day dynamic incubation in PBS at 37 °C (b) in 318-fold and 400-fold magnification, respectively



factor under the same conditions for 15 days. As a control, pure scaffolds were retained.

On PGA/P4HB a decrease in stiffness as expressed by Young's modulus is observed on scaffolds after both static and dynamic incubation in comparison to pure samples. It is assumed that the aqueous environment and the temperature of 37 °C have plasticizing and at a later stage also degradation effects. Their mechanical strength as expressed by UTS decreases significantly after a 15-day incubation under static and dynamic conditions, by 87% and 84% respectively (Fig. 3). The same holds for the elongation at break (Fig. 4), with the strained scaffolds showing an additional decrease (67%) compared to static samples (36%). The altered mechanical behavior of PGA/P4HB scaffolds is elucidated by the increasing degradation rate after 15-day incubation (Fig. 5). E-SEM micrographs agree with those findings revealing disrupted fibers within the scaffolds (Figs. 8b and c). Under straining, fibers appeared to be even more prone to fragmentation, what also correlates to their increased mass loss compared to static samples. Additionally, pronounced fiber alignment as well as disruption of entanglement points provided by the coating towards strain direction may also account for the poor mechanical properties of the strained scaffolds.

The shift towards lower melting temperatures that was observed for PGA after 15 days of both static and dynamic incubation suggests a molecular weight decrease [26]. However, there does not seem to be a difference at the  $T_m$  between the 15-day static and dynamic groups, and it can be therefore hypothesized that no significant differences in molecular weights are present between them. The additional mass loss pronounced in the strained scaffolds may be due to fiber fatigue, which would cause fibers to break and separate from the bulk of the scaffold.

It has to be stressed at this point that properties of PGA/P4HB scaffolds are mainly attributed to the behavior of PGA. The P4HB coating represents only a minor fraction of the composite scaffold as it is added in a 1% (w/v) solution. This can be also confirmed by E-SEM micrographs (Fig. 8).

On PCL scaffolds a decreasing trend on all mechanical properties was exhibited with time of incubation, with an additional decrease on strained samples. In particular, 15-day strained samples showed a much more brittle behavior than statically incubated samples (Fig. 3). The elongation at break decreased by 31% on 15-day static scaffolds, whereas on strained samples a dramatic decrease of 78% was observed (Fig. 4).

No degradation could be determined on this type of scaffold. This is in accordance with the fact that PCL has a relatively low biodegradation rate (>24 months, depending on part geometry, [21, 22]). This fact was also emphasized by DSC as no changes in the melting behavior which would indicate changes in molecular weight were observed, as well as by E-SEM micrographs which showed that nanofibers were still present and not cut or degraded (Figs. 9a and 9b).

The mechanism behind the loss of mechanical properties in electrospun PCL scaffolds which were subjected to cyclic straining seems to be less clear. Incubation under physiological conditions (aqueous environment and temperature) promotes an increase in the polymer free volume and mobility [27], which would be expressed with changes in crystallinity and subsequently in the mechanical behavior. However, loss of mechanical properties was significant only on the 15-day strained samples, whereas the crystallinity increase of the scaffolds did not prove to be statistically significant. We therefore hypothesize that a reorganization of the fibers due to straining might have occurred due to the absence of entanglement points between the fibers and this may account for the brittle behavior in this experimental group.

PCL scaffolds exhibited a big heterogeneity in fiber structure and this may explain the variability in mechanical behavior as observed by the large standard deviations. This indicates that the processing conditions still have to be optimized in order to achieve more reproducible fabrication and thus constant (and better) scaffold properties.

## Conclusions

This study has demonstrated that scaffolds' mechanical properties may be highly influenced by in vitro conditioning parameters. It might not be safe to judge a scaffold by the intrinsic properties of its material(s). These changes should be considered at the application of in vitro conditioning protocols as the scaffolds might not perform as desired and not sustain repetitive loading after a certain point. Additionally, these differences must be accounted for when evaluating the effectiveness of mechanical stimulation on tissue engineered constructs.

Determining the balance point between supports provided by the scaffold and the formation of tissue that has sufficient mechanical integrity to support itself still remains a big challenge in cardiovascular tissue engineering. Future studies will focus on the biomechanics of tissue development and the improvement of in vitro conditioning protocols aiming at better tissue growth. The appropriate scaffold for certain protocols will be selected and a thorough investigation of the contribution of the two components, i.e. scaffold and cells, on the tissue engineered construct's mechanical properties will be made.

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