Structural response of melt-spun poly(3-hydroxybutyrate) fibers to stress and temperature

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ABSTRACT

We have investigated the structural response of melt-spun poly-3-hydroxybutyrate (P3HB) fibers to stress and temperature and its impact on the mechanical properties. Low-stress (<1.6 MPa, 100–130 °C) annealed P3HB fibers showed a considerable viscoelastic behavior and remained ductile up to at least two months. Stress annealing with high weights (>32 MPa), however, lead to fibers with a higher tensile strength (182 MPa) and with a lower elongation at break (22%). These significant differences in the tensile properties are closely related to structural changes, which we have studied with in-situ wide-angle x-ray diffraction (WAXD) and small-angle x-ray scattering (SAXS) experiments. A highly oriented non-crystalline mesophase (P_{nc}) is trapped between orthorhombic α-crystals during high-stress annealing but disappears during low-stress annealing. However, it is possible to restore the mesophase by post-drawing. The viscoelastic hysteresis behavior of low-stress annealed fibers is explained by a reversible transformation of α-crystals into mesophase and back.

1. Introduction

Poly-3-hydroxybutyrate (P3HB) is a thermoplastic polyesteralkanoate (PHA), which is biodegradable and therefore of specific interest for biomedical applications such as tissue engineering scaffolds, bone and cartilage scaffolds, drug delivery, sutures or wound dressing [1–6]. For such future applications, it is crucial to tailor the mechanical properties of P3HB fibers in order to fulfill specific requirements regarding tenacity and elasticity.

In this paper, we study how stress and temperature affect the structure and thus the mechanical properties of melt-spun P3HB fibers. We have obtained stable P3HB monofilaments by an up-scalable melt-spinning method, where the take-up godet was installed at an unusually short distance from the spinneret [7]. These monofilaments consist of a network of longitudinally oriented lamellae (α-crystals) that are embedded in the amorphous phase. The orthorhombic α-crystals are formed by molecular chains that adopt the 2_1 helix conformation [8]. We have previously shown that a highly oriented non-crystalline mesophase (P_{nc}) is trapped between α-crystals [9]. This mesophase consists of stretched tie molecules and leads to a broad equatorial peak in the wide-angle x-ray diffraction (WAXD) patterns [9]. In the past, the mesophase has been found to greatly influence the mechanical properties of P3HB films and fibers, but its exact role in the fiber performance is still a matter of debate. The P_{nc} mesophase is very similar to the commonly denominated β-form phase. The β-form model is based on a paracrystalline (hexagonal [10]) β-form phase, which is formed by stretched molecular chains with zigzag conformation [11,12], whereas the P_{nc} mesophase is non-crystalline and is made of highly oriented stretched chains with varying conformations. We have previously seen that this mesophase is reversibly transformed from and into α-crystals during cyclic tensile loading [9]. Arguments that speak for the existence of a mesophase in our melt-spun P3HB fibers are given in our previous publication [9]. In the remainder of this manuscript, we refer to this phase therefore as mesophase instead of β-form phase.

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Studies about the effect of drawing and annealing on the structure and mechanical properties of melt-spun P3HB fibers that are directly drawn after extrusion are scarce \cite{7,13}. A few other studies have been reported on amorphous melt-spun fibers where the extrudate was quenched into an ice water bath followed by a one-step or two-step drawing procedure \cite{14-17}. Our group and Yamane et al. have shown that stress annealing can improve the tensile strength in melt-spun P3HB fibers \cite{7,13}. Yamane et al. attributed the increase in tensile strength to that stress annealing can improve the tensile strength in melt-spun P3HB fibers quenched into an ice water bath followed by a one-step or two-step annealing process. Reported on amorphous melt-spun fibers where the extrudate was drawn after extrusion are scarce \cite{7,13}. A few other studies have been explored on the structural response of melt-spun P3HB fibers to low-stress annealing (\( \leq 16 \) MPa) and to high-stress annealing (\( \geq 32 \) MPa) with in-situ wide-angle x-ray diffraction (WAXD) and small-angle x-ray scattering (SAXS) experiments. To the best of our knowledge, we report for the first time a viscoelastic behavior of low-stress annealed P3HB fibers. Similar viscoelastic behaviors have been previously reported for other biomaterials such as \( \alpha \)-keratin fibers or horsehair \cite{23-25}.

Our x-ray results from in-situ drawing and stress-annaling experiments combined with molecular dynamics simulations and previous observations from cyclic drawing studies \cite{7,9} enable us to present a structural model of melt-spun P3HB fibers, which explains the growth of the mesophase during tensile drawing and its disappearance during annealing with low-stress. Furthermore, the mechanisms responsible for the viscoelasticity of low-stress annealed P3HB fibers are elucidated.

2. Materials and methods

2.1. Materials

The fiber used for this study was melt-spun from modified P3HB (density 1.2 g/cm\(^3\)) provided by Biomer (Krölling, Germany) on a customized pilot melt-spinning plant originally built by Fourné Polymertecnich (Alfter-Impkevoven, Germany). A take-up godet was mounted at an unusually short distance (0.75 m) from the spinneret. This godet prevented the crystallization of the extrudate in a randomly oriented state. The fiber was melt-spun with a draw ratio of seven from ready-to-use modified P3HB pellets. The P3HB pellets contained a nucleating agent (boron nitride (BN)) and a plasticizer (tri-n-butyl citrate (TBC)) as well as various other processing aids including poly-\( \varepsilon \)-caprolactone (PCL). The fiber diameter is about 90 \( \mu \)m. More detailed information about materials, melt-spinning parameters, and the properties of the P3HB fiber can be found in our previous publications \cite{7,9}.

2.2. Annealing procedures

Annealing of the aged P3HB fiber (seven years) was conducted under various loads (1 g, 10 g, 20 g, 25 g) corresponding to 1.6, 16, 32, 40 MPa at different temperatures (60, 80, 100, 115, 130 °C) in a furnace with hot air circulation. The fibers with low loads (1.6, 16 MPa) were kept at all temperatures for 60 min. For the high loads (32, 40 MPa) the fibers were also kept for 60 min in the furnace for temperatures \( \leq 100^\circ \)C. Above 100 °C the fibers could only be kept for 5 min at 105 °C in the furnace, because longer annealing times or higher temperatures caused breakage of the fibers. The length of each monofilament was measured at room temperature with a weight of 1 g before and after annealing in order to determine the change in filament diameter and thus the linear mass density.

2.3. Tensile measurements of aged and stress-annealed fibers

We have performed tensile tests after aging and annealing. The load-strain behavior of the fibers was evaluated using the Textechno STA-TIMAT ME+ (Herbert Stein GmbH, Germany) tensile tester with a 10 N load cell, following ASTM D2256. The tensile tests of the monofilaments were performed with a typical test length of 100 mm and with a constant elongation rate of 100 mm/min. Hysteresis measurements on low-stress annealed fibers were performed on a Zwick Z100 (Zwick Roell GmbH, Germany) testing machine with a test length of 100 mm, a 10 N load cell and a constant elongation/contraction rate of 100 mm/min. The time between consecutive hysteresis loops was 20 min.

2.4. In-situ laboratory WAXD and SAXS of fibers during stress annealing

WAXD and SAXS patterns during in-situ annealing of three to four years aged fibers were recorded on a Bruker Nanostar U diffractometer (Bruker AXS, Germany) with Cu K\( \alpha \) radiation (\( \lambda = 1.5419 \) Å) and a VANTEC-2000 MikroGap area detection system. A beam defining pinhole of 300 \( \mu \)m was used. The WAXD and SAXS measurements were performed in two separate experiments with distances of 17.1 cm and 96.3 cm, respectively, between sample and active detector area. The heating stage H+300 (Bruker AXS, Germany) of the Nanostar diffractometer was used in order to study the effect of annealing. Single filaments were mounted on a custom-made fiber holder and different weights were attached at the end of the filaments in order to study the combined influence of heat and tension.

Two WAXD and SAXS experiments were performed with the in-situ heating stage: (A) The effect of low-stress annealing on the structure was studied by attaching a very small weight of 85 mg (0.14 MPa) to the end of the filament in order to keep the fiber straight during the measurement. (B) The influence of high-stress annealing on the structure was studied by attaching a weight of 20 g (32 MPa) to the filament. The stage with the filament was heated to various temperatures with 10 °C/min, and SAXS/WAXD patterns corresponding to the respective temperatures were recorded for typically 30 min or longer. During data collection, the temperature was kept constant by the heating stage control unit. The tables with stress/temperature conditions and exposure times of the individual measurements of the WAXD and the SAXS experiment, respectively, can be found in the data in brief article \cite{26}.

The recorded WAXD/SAXS patterns were analyzed with the evaluation software DIFFRAC.EVA (version 4.2., Bruker AXS, Germany) and specially developed Python codes. Long-spacings, coherence lengths and lamellar sizes were calculated by analyzing meridional and transversal areas of the SAXS pattern \cite{27} (data in brief article \cite{26}). The intensities of all WAXD/SAXS patterns were corrected for exposure time and the thinning or thickening of the fibers from measured changes in the filament length.

2.5. In-situ synchrotron WAXD and SAXS of fibers during post-drawing after annealing

In-situ WAXD and SAXS measurements were performed at the eSAXS beamline at the Swiss Light Source of the Paul Scherrer Institute in Switzerland. The drawing at room temperature was performed using a TS 600 tensile stage (Anton Paar GmbH, Austria) with a 5 N load cell. A pre-annealed single filament was glued on top of supports and held by tensile stage grips. The single filament was elongated with an elongation rate of 0.5 mm/min until breakage while exposing the filament to the synchrotron x-ray beam every 30 s for 0.5 s. A vertically oriented Pilatus 300 K detector (Dectris LTD, Switzerland) was used to capture the equatorial range (0.55–2.75 Å\(^{-1}\)) of the WAXD patterns. Simultaneously, the SAXS patterns were measured with a Pilatus 2M detector \cite{28}. A 2 m long flight tube was positioned in-between the SAXS detector and the drawing stage. The sample to detector distance was 2.139 m. The x-ray energy was 11.2 keV and the vertical beam spot size was about 25 \( \mu \)m.

2.6. Molecular dynamics simulations

Molecular dynamics simulations were performed in order to simulate...
the transformation of stretched tie molecules to helical chains in α-crystals. The initial configuration of chains in the α-crystal was taken from the previously published structures by Wang et al. [8]. The General AMBER Force Field (GAFF) is used to model the non-bonded and bonded interactions in P3HB chains [29]. In this model, the standard 12-6 Lennard-Jones potential and electrostatic interactions for non-bonded interactions are used in combination with bond, angle, and dihedral potentials for bonded interactions [9,30,31]. All simulations were carried out in NVT ensemble at 298 K.

3. Results and discussion

3.1. Tensile properties of aged and stress-annealed fibers

We have stress annealed the long-term aged (7 years) fiber at different temperatures with different applied weights. As mentioned in the materials section, the length of each monofilament was measured at room temperature with a weight of 1 g before and after annealing in order to determine the change in filament diameter and thus the linear mass density. We have observed a shrinkage of the low-stress annealed fibers and an elongation of high-stress annealed fibers. The calculated linear mass densities of annealed fibers are given in the data in brief article [26]. Fig. 1 shows the measured tensile strength (Fig. 1a), elongation at break (Fig. 1b) and toughness (Fig. 1c) as a function of the annealing temperature for all stress annealing conditions. Error bars are calculated standard deviations from the number of measurements ranging from 2 to 10, depending on the annealing condition. The corresponding tables with the exact values are given in the data in brief article [26]. The reference values of the not-annealed long-term aged (7 years) fiber are shown as dashed black lines in Fig. 1. The achieved tensile strengths are comparable to wool fibers [32].

Annealing with high stress leads to a slight reduction in elongation at break but to an increase in tensile strength. At best, we have obtained an ultimate tensile stress of 182 MPa for the stress-annealed fiber at 105 °C with 40 MPa, which corresponds to a relative increase of 23% compared to the untreated fiber. The elongation at break and toughness are, however, the highest for low-stress annealed fibers at 115 °C, which is accompanied by a loss in tensile strength. Since toughness is a measure of the ability of the material to absorb energy, these low-stress annealed fibers could be used in biotextiles as shock-absorbing materials.

A selection of corresponding stress-strain curves of stress-annealed (1.6, 16 and 40 MPa) fibers is shown in Fig. 2a including the curves of the as-spun fiber (black full curve) and of the long-term aged fiber (black, dash-dotted curve), which was stored at 23 °C for seven years. The fiber exhibited a 5% improvement of tensile stress after the first month of aging [7] and remained stable within these seven years. Interestingly, the low-weight (1.6 MPa) annealed fibers (at 115 °C) have a very high elongation at break (91%) due to a pronounced deformation plateau. All of the low-stress annealed fibers (at 100–130 °C) remained elastic and stable up to at least two months. Nine consecutive hysteresis cycles of the fiber pre-annealed at 115 °C with 1.6 MPa are shown in Fig. 2b. The tensile stress was calculated using the diameter of the initial fiber before the first cycle, thus the maximum stress is decreasing from cycle to cycle due to the thinning of the fiber. During each cycle a plastic deformation remains, which is apparent from the starting tensile strain of each cycle. Similar viscoelastic behavior has been previously observed for example in α-keratin fibers, where the viscoelasticity has been attributed to a transformation of helical molecules from the α-phase to stretched molecules in β-sheets and back [23–25].

These significant differences in the mechanical properties (Fig. 2) are of great interest for biomedical or biotextile applications. Depending on the application, the fibers have to meet different demands regarding mechanical properties. For example, some applications are based on biodegradable shock-absorbing ductile textiles, while others need high-tenacity fibers.

Previously, the deformation mechanism of thermoplastics has been described with a Gaussian model developed by Haward and Thackray [33–36]. The model is based on a Hookean spring, which is in series with a parallel circuit of a dashpot and another rubbery spring (inset Fig. 3a). The Hookean spring and the dashpot relate to the movement of the Hookean spring and the dashpot relate to the movement of the distorted coil, whereas the Langvin equation applies when chains approach their maximum extension between

Fig. 1. (a) Tensile strength, (b) elongation at break and (c) toughness plotted against the annealing temperature for different stress values. The dashed black lines are the reference values of the long-term aged fiber.
entanglements [33]. Fig. 3a shows the data from Fig. 2a converted into Gaussian plots of true stress, $\sigma_{true}$ vs. $\lambda^{2}-1/\lambda$. Three regions can be distinguished in the stress-strain curves: An initial region, I, with a steep slope, where the deformation is approximately following Hooke’s law. A second middle region, II, and a third strain hardening region, III. Since both regions, II and III, show a rather linear behavior with $\lambda^{2}-1/\lambda$, we have extracted two moduli from the Gaussian plots, one from the middle region, $G_{II}$, and one from the region at large strains, $G_{III}$. The elastic modulus E, from the first region I, was not extracted, since not many data points are available in this region. The $G$ moduli are plotted against annealing stress values in Fig. 3b. The dashed horizontal lines correspond to the reference values of the long-term aged fiber (7 years). The rubber elastic modulus $G_{II}$ from the middle region is large (524 MPa) for the long-term aged fiber (dashed black line). Low-stress annealing with 1.6 MPa at 115 °C leads to a significant decrease of this modulus to 21 MPa, whereas high-stress annealing with 40 MPa at 105 °C leads to an increased $G_{II}$ modulus of 558 MPa. In contrast, the strain hardening modulus $G_{III}$ is small (119 MPa) for the long-term aged fiber (green dashed line). A similar value (117 MPa) is found for the low-stress annealed fiber. High-stress annealing is, however, significantly increasing the strain hardening modulus up to 161 MPa. Various structural factors affect these $G$ moduli and are discussed in more detail in section 3.4.

These differences in the mechanical properties of annealed fibers are closely related to structural changes that occur during the annealing procedures. We have measured WAXD and SAXS patterns during in-situ annealing in order to elucidate the structural response to different stress and temperature conditions (sections 3.2-3.3). The occurrence of three regions in the Gaussian plots (Fig. 3a) indicate that several phases exist in the fibers and that different types of structural changes must occur during drawing. To further elucidate such structural changes, we have performed in-situ synchrotron WAXD and SAXS measurements of a low stress annealed fiber during drawing (section 3.4).

3.2. In-situ laboratory WAXD during stress-annealing

Fig. 4 shows in-situ WAXD patterns taken at room temperature before annealing under low tension (0.14 MPa, Fig. 4a), during annealing at 103 °C summed over 1 h (Fig. 4b) and after annealing cooled to room temperature and summed over 2 h (Fig. 4c). The same measurements for the fiber during high-stress annealing with 32 MPa are shown in Fig. 4d–f. The reflections from the polymeric processing aid poly-ε-caprolactone (PCL) are indicated with a star. PCL has its (110) equatorial peak at this exact location [37]. In our previous publication, we have also seen the (200) PCL peak, which is located next to the (110) peak of PCL in WAXD patterns with a larger angular range [7]. Some reflections from P3HB are labelled according to the orthorhombic α-P3HB unit cell (a = 5.76 Å, b = 13.20 Å and c [fiber axis] = 5.96 Å) [8].

In the first experiment (low-stress, 0.14 MPa), the peak attributed to the PnH mesophase disappears upon heating, whereas in the second experiment (high-stress, 32 MPa), the PnH peak remains due to the applied tension.

In order to better visualize the dependency of the PnH peak intensity on temperature, we have extracted equatorial profiles from the equatorial sectors with opening angles of 20°. The occurrence of three regions in the Gaussian plots (Fig. 3a) indicate that several phases exist in the fibers and that different types of structural changes must occur during drawing. To further elucidate such structural changes, we have performed in-situ synchrotron WAXD and SAXS measurements of a low stress annealed fiber during drawing (section 3.4).
background corrected equatorial profiles are shown in Fig. 5 for different temperatures during low-stress annealing (Fig. 5a) and high-stress annealing (Fig. 5c). The respective peak intensities of equatorial α-reflections and of the P_{nc} mesophase are shown in Fig. 5b and d as a function of temperature.

The P_{nc} peak clearly disappears above 80 °C during low-stress annealing (Fig. 5b), and the α-phase peaks are increasing in intensity. This observation is in analogy to previously reported structural changes in cold-drawn P3HB films during annealing, where the mesophase starts to disappear around 110 °C [20], thus at slightly higher temperatures than in our fibers. In contrast, the P_{nc} peak intensity stays constant in the high-stress annealed fiber (Fig. 5d), but the equatorial α-peak intensities are also strongly increasing.

As argued in our previous publication, the broad P_{nc} peak originates from a highly-oriented non-crystalline mesophase, which is mainly trapped in between α-crystals [9]. The mesophase P_{nc} consists of highly oriented tie molecules between α-crystals. Thus, during low-stress annealing, the stretched molecules from the P_{nc} mesophase can recoil to helical shapes and contribute to the α-crystals. During high-stress annealing, however, the stretched chains remain elongated since a high weight is attached to the fiber and the P_{nc} mesophase is thus not disappearing.

In order to quantify the content of the individual phases in the fibers at different annealing temperatures, we have summed the Lorentz and polarization corrected [27] WAXD patterns along the azimuth (360° in φ, radial profiles). The background arising from the amorphous phase
and the (021) peak was subtracted from these radial profiles following the same procedure as previously described [9]. The resulting integrated (020) and (110) annuli and the $P_{nc}$ peak are shown in Fig. 6. These three peaks were fitted with Pearson VII functions and the calculated percentages from the peak areas are given in the insets of Fig. 6. During low-stress annealing, the $\alpha$-phase content increases from 25% to 45%, whereas the $P_{nc}$ phase content decreases from 15% to 0%. We conclude that the stretched molecules from the $P_{nc}$ phase recoil and transform into helical conformations, leading to a growth of the $\alpha$-crystals. The latter also explains the increase in the equatorial $\alpha$-peak intensities (Fig. 5b).

Interestingly, the $\alpha$-phase content of the filament under high tension (Fig. 6b) does not significantly change (red dots) during annealing, even though the equatorial $\alpha$-peak intensities strongly increase (Fig. 5d). The latter suggests that the intensity increase in the equatorial $\alpha$-peaks (Fig. 5d) is coming mainly from an increased orientation of $\alpha$-crystals, which we have also confirmed by analyzing azimuthal profiles (data in brief article [26]). Furthermore, the $P_{nc}$ mesophase content is hardly affected by the annealing procedure (Fig. 6b, green diamonds). We conclude that the applied tensile stress prevents the stretched $P_{nc}$ molecules from transforming into helical chains, and thus $\alpha$-crystals are hardly growing.

![Fig. 6](image)

Fig. 6. Difference intensity, $\Delta I$, between integrated intensity along the azimuth (360° in $\phi$, radial profiles) and intensity arising from the amorphous phase and (021) peak [9]; Applied tensile stress: 0.14 MPa (a) and 32 MPa (b) for different annealing temperatures. The sequence of the temperature steps follows the order given in the legend from top to bottom. Inset: The percentage of amorphous phase and (021) peak intensity is shown as grey squares. The crystallinity of $\alpha$-form (oriented and unoriented) is shown as red circles. The percentages of the $P_{nc}$ phase are shown as green diamonds. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

### 3.3. In-situ laboratory SAXS during stress-annealing

We have also measured SAXS during in-situ stress-annealing in order to obtain information about structural changes to crystal sizes and long spacings. SAXS patterns for the fiber with a small applied pre-tension of 0.14 MPa are shown in Fig. 7a–c, and for a tension of 32 MPa in Fig. 7d–f.

Meridional profiles were extracted from the SAXS images by averaging the intensities in the area between vertical black dashed lines and transversal profiles were extracted from the areas between the horizontal dotted lines. The profiles are shown in the data in brief article [26] for all temperatures. The long-spacings, crystal sizes and coherence lengths are extracted from the meridional and transversal profiles (data in brief article [26]). Two symmetric reflections, (1) and (2), are clearly visible for the initial starting temperature 25 °C. Peak (1) disappears above 45 °C, whereas (2) becomes sharper, more intense and shifts to smaller diffraction angles. We attribute peak (1) to PCL, a processing aid, since the peak is only present in fibers that contain PCL. Furthermore, PCL has a low melting point at around 60 °C, which is close to the observed melting point of peak (1) under tension. The long-spacing, $L$, is about 14.1 nm (at 0 MPa, 25 °C) and 15.3 nm (at 32 MPa, 25 °C), respectively, which agrees well with previously published long-spacings for PCL crystals [38,39]. The second reflection (2) arises from P3HB $\alpha$-crystals.

The extracted long-spacings, coherence lengths and crystal sizes (lamellae stack diameters) for the second reflection are shown in Fig. 8 as a function of the temperature sequence.

Corresponding tables with the specific values are given in the data in brief article [26]. The bottom of Fig. 8 shows the pairs of coherently diffracting P3HB $\alpha$-crystals with the corresponding crystal sizes (lamellae diameters) and long-spacings before, during and after annealing. The mesophase is mainly located between the $\alpha$-crystals before annealing but also exists in the amorphous phase. We explain the observed changes in crystal size, long-spacing and coherence length during annealing with the following models:

**Case A, 0.14 MPa:** The non-annealed as-spun fibers at 25 °C have $\alpha$-crystals that are elongated perpendicular to the fiber axis. The coherence length around 17.4 nm indicates that in average two lamellae are typically coherently aligned along the fiber direction with a long spacing of about 7.8 nm (bottom of Fig. 8a). At high temperatures (115 °C), the crystals grow along the fiber direction as well as perpendicular to it, which is reflected by the increase in the long-spacing and crystal size between 90 and 115 °C. The $P_{nc}$ phase, which is located in between $\alpha$-crystals, vanishes (seen by WAXD). This means that the stretched chains of the $P_{nc}$ phase recoil into helical conformations during heating, which results in almost no space between coherently aligned crystal pairs. After the cool-down, these large crystals remain. The elastic behavior of low-stress annealed fibers is thus related to the structure after annealing, which is a network of amorphous phase and $\alpha$-phase without $P_{nc}$ mesophase. During the tensile testing of low-stress annealed fibers, this network structure is changing, which is discussed in subsection 3.4.

**Case B, 32 MPa:** The crystals do not significantly grow during the annealing, but they reorient, leading to an increased intensity in the equatorial $\alpha$-crystal WAXD reflections and also in the SAXS reflections. An increase in the coherence length and in the long spacing is observed (Fig. 8b). The $P_{nc}$ phase, which is located in between $\alpha$-crystals, remains (seen by WAXD) since the stretched chains are forced to stay in a stretched conformation by the applied tension (bottom of Fig. 8b).

### 3.4. Time-resolved synchrotron WAXD and SAXS during in-situ post-drawing

In order to investigate the structural changes during post-drawing of low-stress pre-annealed fibers at room temperature, we have performed in-situ WAXD and SAXS at the synchrotron. Additional WAXD/SAXS
results of the in-situ drawing of high-stress, low-stress annealed and untreated aged fibers are shown in the data in brief article [26].

Fig. 9a shows the Gaussian plot (Haward-Thakray curve) of a low-stress annealed fiber (annealed at 115 °C, 1.6 MPa) with the measured equatorial WAXD patterns as insets. The extracted $G_{II}$ and $G_{III}$ are slightly lower than in Fig. 3 since the fiber is glued on a holder on two sides, which might have led to slight errors in the absolute true stress values. The dark blue vertical line in the WAXD patterns is coming from the gap between two detector modules. In total, 24 images were taken with an interval of 30 s during the drawing, and some image numbers are indicated in the figure. Fig. 9b shows the radially integrated WAXD patterns. At the beginning of the measurement (region I), no $P_{cc}$ peak is visible in the WAXD pattern, which confirms the previous observations from in-situ WAXD measurements in the laboratory, where the $P_{cc}$ peak vanished during the annealing. Interestingly, upon drawing, a $P_{cc}$ peak appears mainly during the deformation plateau (region II). Fig. 9c shows corresponding SAXS images, with image numbers and corresponding acquisition time.
central diffuse scattering (CDS) changes from a diamond shaped scattering to an elongated ellipsoidal diffuse scattering along the fiber axis and is strongly increasing in intensity during drawing. This CDS suggests that uncorrelated inhomogeneities form, which are elongated perpendicular to the fiber axis. Upon further drawing, these inhomogeneities also grow along the fiber axis, leading to a sharpening of the diffuse scattering along the meridian towards the direct beam. Most likely, these inhomogeneities are arising from the P\textsubscript{nc} mesophase, since the diffuse scattering in SAXS starts to occur at the same time as the P\textsubscript{nc} appears in the WAXD pattern. However, the CDS is located close to the direct beam, which means that the inhomogeneities have to be quite large (>12 nm) along the fiber axis. This suggests that in these fibers the mesophase may also be transformed from the amorphous part. Thus, the P\textsubscript{nc} is not only located in-between the lamellae, but also between pairs of lamellae. Interestingly, fibers that were annealed with a high weight do not show such a CDS (data in brief article [26]).

In region I, both the strong ellipsoidal CDS in the SAXS as well as the P\textsubscript{nc} peak in the WAXD are not present. However, in region II, parts of the helical chains start to be pulled out of the \(\alpha\)-crystals and become stretched and transform into the P\textsubscript{nc} mesophase. The newly grown parts of the \(\alpha\)-crystals during the low-stress annealing have a high degree of perfection and are more easily transformed into the P\textsubscript{nc} mesophase than the surficial parts of the \(\alpha\)-crystals from high-stress annealed fibers. This observation is confirmed by the small rubber elastic modulus \(G\textsubscript{II}\), the slope of the middle region in the Gaussian plots and reflects the easiness to pull-out chains from \(\alpha\)-crystals and to stretch parts of the entangled amorphous phase. The growth of the P\textsubscript{nc} mesophase is thus mainly happening in region II. Our hypothesis is that tie molecules unravel and recoil during low-stress annealing, leading to larger crystals. The surficial parts of these crystals can thus be more easily transformed back into the P\textsubscript{nc} mesophase upon stretching, since no entangled tie molecules hinder the uncoiling. However, such entangled tie molecules might exist in high-stress annealed fibers. In region III, the chains from the mesophase become even further stretched. The slope of the Gaussian plots in region III is the strain hardening modulus \(G\textsubscript{III}\), which reflects the easiness to further stretch chains of the amorphous phase and the P\textsubscript{nc} mesophase. \(G\textsubscript{III}\) is increasing with increasing annealing stress. During high-stress annealing, chains are already in a highly-stretched conformation and thus it is harder to further elongate the chains, leading to a higher strain hardening modulus. In the literature, a large strain hardening modulus has also often been correlated with a higher density of entanglements [36].

These results suggest that the viscoelasticity can be explained by a partially reversible transformation of \(\alpha\)-crystals and amorphous phase into P\textsubscript{nc} phase and back. Similar viscoelastic properties have been observed in other biomaterial based fibers such as Keratin or Hagfish fibers, where the structural changes are explained by an \(\alpha\)-crystal to \(\beta\)-sheet transformation [23–25].

3.5. Molecular dynamics simulations

In order to confirm that stretched tie molecules can recoil into helical molecules of an \(\alpha\)-crystal during low-stress annealing, we have performed molecular dynamics (MD) simulations. A simulation box with a representative \(\alpha\)-crystal, containing 48 P3HB chains, was used for this
purpose (Fig. 10a). Each chain consisted of 46 monomers and the simulation box had lateral periodic boundary conditions. For the starting simulation condition (initial crystal), the atoms in a slab of 11.7 Å in thickness (shown in red in Fig. 10a) were allowed to move and equilibrate for 2 ns. After that, the upper section with the α-crystal was moved upwards with a constant velocity to induce a stretch on the chains in the slab, resulting in the P$_\alpha$ mesophase (stretched configuration). The final thickness of the slab/mesophase was $\sim$27.7 Å. Depending on the stretch factor, different chain conformations can be obtained in these simulations [9,11,27,40]. The chains were allowed to equilibrate for 5 ns. The third part of the simulation consisted of returning the stretched structure to its initial thickness by moving the upper section back to its original position during a dynamic run of $\sim$5 ps, followed by 5 ns equilibration time (compressed configuration). The atomic positions in the slab were averaged during each equilibrium run in this three-step cycle (initial, stretched, compressed), and are presented in Fig. 10b–d.

The equilibrated chains in the initial slab adapt helical conformations (Fig. 10b). However, upon stretching the chains transform into a mesophase and dramatically deviate from this helical conformation (Fig. 10c). The simulations confirm that the mesophase chains can potentially recoil into helical conformations when the tension is released (Fig. 10d). Note that the compression mechanism applied here is only reflecting the potential recrystallization that happens during low-stress annealing and is thus not a definitive proof on its own. However, the MD simulation results, together with the experimental evidence from x-ray analysis, strongly support the concept of the recoiling of stretched tie molecules from the P$_\text{nc}$ mesophase to α-crystals.

4. Conclusions

We have investigated the structural response of melt-spun P3HB fibers to stress and temperature and its impact on the mechanical properties. Tensile tests have revealed that very different mechanical properties can be obtained depending on the pre-annealing conditions (applied stress and temperature): A) Low-stress annealed fibers are viscoelastic with elongations at break of up to 91%. After two months of aging, such fibers remained elastic, and future tests will show if further aging will affect the viscoelastic behavior. B) High-stress annealed fibers have higher tensile strength (max. 182 MPa) but lower elongation at break (22%). Thus, high-stress annealing is a method that can be used to increase the tensile strength of P3HB fibers. However, further investigations are needed in order to test different stress annealing methods during the online drawing of P3HB fibers. The significant differences in the mechanical properties of low-stress and high-stress annealed fibers are closely related to structural changes that occur during the annealing procedures. In-situ WAXD and SAXS measurements during low-stress and high-stress annealing have shown that in case (A), a secondary crystallization occurs, leading to a growth of α-crystals while the mesophase disappears. The applied heat transfers sufficient energy to the stretched chains of the trapped mesophase to allow the chains to overcome the lateral van der Waals forces and crystallize into helical chains, which have the lowest potential energy. During post-drawing of these low-stress annealed fibers, helical molecules from α-crystals and chains from the amorphous phase transform into elongated molecules, giving rise to an enhanced equatorial P$_\text{nc}$ peak in the WAXD pattern and a strong central diffuse scattering in SAXS patterns. In high-stress annealed fibers (B), however, the applied tension prevents the stretched chains in the mesophase from recoiling into helical chains but promotes the orientation of α-crystals, leading to an increased tensile strength.

The results of this in-situ low-stress and high-stress annealing WAXD/SAXS study, combined with the results of the tensile tests and molecular dynamics simulations, support the concept that the broad equatorial WAXD peak can be attributed to a non-crystalline highly oriented mesophase (P$_\text{nc}$) that is trapped between α-crystals. This mesophase is made of highly stretched tie molecules of different conformations that are arranged in an irregular manner on the ab-plane. We have previously shown that the α-crystals partially transform into the P$_\text{nc}$ mesophase when stress is applied without heating [9]. This transformation was found to be reversible to a high degree. Here, we have now shown that this reversible transformation can be further promoted by low-stress annealing. During low-stress annealing the mesophase vanishes, and thus the fiber can absorb more energy, leading to a significant viscoelastic behavior, where a partially reversible transformation from α-crystals and amorphous phase to P$_\text{nc}$ mesophase and back occurs. Such mesophases with load-bearing tie molecules play an important role in many semi-crystalline polymers and need to be further investigated.

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![Fig. 10.](image_url)

(a) Atomicistic representation of P3HB chains in MD simulations of the stretching and relaxation of the α-crystal. Average conformations of the chains in the slab in the initial crystal (b), stretched configuration (c), and compressed configuration (d). In parts b–d, the carbon and oxygen atoms are shown as blue and red spheres, respectively. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)
Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

CRediT authorship contribution statement

Edith Perret: Software, Validation, Investigation, Data curation, Writing - original draft, Visualization. Felix A. Reifler: Validation, Investigation, Data curation, Writing - review & editing. Ali Gooneie: Software, Methodology, Writing - review & editing. Kang Chen: Investigation, Writing - review & editing. Figon Sell: Investigation, Writing - review & editing. Rudolf Hufenus: Supervision, Project administration, Writing - review & editing.

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Appendix A: Supplementary data

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