Structural Evolution of PCL during Melt Extrusion 3D Printing

DOI:

[10.1002/mame.201700494](https://doi.org/10.1002/mame.201700494)

Document Version

Accepted author manuscript

[Link to publication record in Manchester Research Explorer](#)

Citation for published version (APA):

Mirihanage, W., Da Silva Bartolo, P. J., Poologasundarampilla, G., Hinduja, S., Vyas, C., Liu, F., & Pape, I. (2018). Structural Evolution of PCL during Melt Extrusion 3D Printing. *Macromolecular Materials and Engineering*, 1700494-1700500. <https://doi.org/10.1002/mame.201700494>

Published in:

Macromolecular Materials and Engineering

Citing this paper

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Macromolecular Materials and Engineering

STRUCTURAL EVOLUTION OF PCL DURING MELT EXTRUSION 3D PRINTING

--Manuscript Draft--

Manuscript Number:	name.201700494R2
Full Title:	STRUCTURAL EVOLUTION OF PCL DURING MELT EXTRUSION 3D PRINTING
Article Type:	Communication
Section/Category:	
Keywords:	Additive biomanufacturing; crystallization; in situ X-ray diffraction; screw-assisted melt extrusion.
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Abstract:	Screw-assisted material extrusion technique has been developed for tissue engineering applications to produce scaffolds with well-defined multi-scale microstructural features and tailorable mechanical properties. In this study, in situ time-resolved synchrotron diffraction was employed to probe extrusion-based 3D printing of polycaprolactone (PCL) filaments. Time-resolved X-ray diffraction measurements revealed the progress of overall crystalline structural evolution of PCL during 3D printing. Particularly, in situ experimental observations provide strong evidence for the development of strong directionality of PCL crystals during the extrusion driven process. Results also show the evidence for the realization of anisotropic structural features through the melt extrusion-based 3D printing which is a key development towards mimicking the anisotropic properties and hierarchical structures of biological materials in nature, such as human tissues.
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Question	Response
Please submit a plain text version of your cover letter here.	Dear Prof. Kirsten Severing,
Please note, if you are submitting a revision of your manuscript, there is an opportunity for you to provide your responses to the reviewers later; please	We are submitting this original research communication entitled "Structural evolution of PCL during melt extrusion 3D printing" for your consideration. We confirm that this work is original and has not been published nor is it currently under consideration for publication elsewhere.

do not add them to the cover letter.	<p>This communication is one of the first studies investigating the evolution of crystallinity and crystal orientation during melt extrusion 3D printing via in situ time-resolved synchrotron based X-ray diffraction. We consider the results highly relevant to Macromolecular Rapid Communications readers and this communication timely due to its relevance for controlling the anisotropic structural features of 3D printed scaffolds for tissue engineering applications, which is a rapidly growing research field. We believe that Macromolecular Rapid Communications is the most suitable journal for this communication as it publishes high quality original research in polymer science, ranging from chemistry and physics of polymers in material science and life sciences. Also, the viewing time and average publication time are relatively short, good for fast publication of high priority results.</p> <p>We would also like to emphasise that there are no conflicts of interest to disclose.</p> <p>Sincerely, Paulo Bartolo Professor, School of Mechanical, Aerospace and Civil Engineering University of Manchester</p>
Do you or any of your co-authors have a conflict of interest to declare?	No. The authors declare no conflict of interest.

Structural evolution of PCL during melt extrusion 3D printing^a

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Screw-assisted material extrusion technique has been developed for tissue engineering applications to produce scaffolds with well-defined multi-scale microstructural features and tailorable mechanical properties. In this study, in situ time-resolved synchrotron diffraction was employed to probe extrusion-based 3D printing of polycaprolactone (PCL) filaments. Time-resolved X-ray diffraction measurements revealed the progress of overall crystalline structural evolution of PCL during 3D printing. Particularly, in situ experimental observations provide strong evidence for the development of strong directionality of PCL crystals during the extrusion driven process. Results also show the evidence for the realization of anisotropic structural features through the melt extrusion-based 3D printing which is a key development towards mimicking the anisotropic properties and hierarchical structures of biological materials in nature, such as human tissues.

^a **Supporting Information** ((bold)) is available online from the Wiley Online Library or from the author.

1. Introduction

Regenerative medicine and tissue engineering are rapidly expanding fields that aim to repair, replace, and regenerate damaged cells, tissues, and organs ^[1-3]. Different methods have been explored including cell therapy, the use of scaffolds or tissue engineered scaffolds with cells and the use of cell-laden constructs ^[4]. However, the scaffold-based approach is very frequently used in tissue engineering. Scaffolds are three-dimensional biocompatible and biodegradable porous structures that provide substrates for cell attachment, differentiation and proliferation ^[5,6]. Scaffold design also requires the control of material properties, surface topography, chemistry and stiffness that significantly influence biological processes ^[7]. Furthermore, precise spatial positioning of different materials is required during the scaffold fabrication process. This has become possible with the development of additive biomanufacturing techniques which allow a wide variety of materials to be used including biological materials such as cells^[8-10]. Different additive biomanufacturing strategies, such as powder bed fusion, stereolithography, extrusion-based and inkjet printing processes have been explored to produce scaffolds with precise control over material deposition and construct architecture for a variety of applications including bone, cartilage, nerve and skin ^[11-18]. Extrusion-based techniques are widely utilized due to the versatility and simplicity of the technique which enables a variety of materials to be processed, the ability to print in a cell-friendly environment as well as the feasibility for controlled high precision deposition. Different extrusion-based techniques have been developed including the use of pneumatic or mechanical assistance methods, utilizing a piston or a screw, to apply the extrusion force ^[19]. The screw-assisted method is ideal for producing scaffolds to realize bone regeneration as it allows precise deposition, high deposition velocity, improved material mixing, being able to manipulate materials with high viscosity ^[20].

PCL is one of the most commonly used synthetic polymers for tissue engineering and regenerative medicine applications ^[21-23]. This is due to the advantageous properties of PCL which make it suitable for tissue engineering applications. These include biocompatibility with it being utilized in many FDA approved devices, long-term biodegradability, ease of processing due to the low melting temperature (~58-60°C), suitable rheological and viscoelastic properties, and thermal stability ^[24-26]. PCL and PCL based composites have been explored in a range of applications in tissue engineering ^[27-30]. Different manufacturing techniques including additive biomanufacturing and conventional methods have been employed to fabricate PCL structures ^[31-33]. However, the effects of fabrication processing parameters have on the degree of crystallinity, crystallite size, and crystal orientation of the polymers, have not been widely researched. Crystallinity and crystal orientation ^[34] of synthetic polymers are crucial since they can directly affect the scaffold properties having an effect on the cellular phenotype and cellular spreading ^[35]. In addition, the molecular weight of PCL influences the crystallization kinetics ^[36], and also the bulk material stiffness ^[37] can influence stem cell differentiation. However, structure evolution during filaments formation is rarely studied; probably because of its dynamic nature and the lack of experimental tools for such studies. In order to realize the full potential of 3D extrusion printing and in specific to engineer the properties of the printed constructs, it is essential to understand the development of microstructure during extrusion-based printing processes.

This paper presents one of the first studies to investigate the evolution of crystallinity and crystal orientation during melt extrusion via in situ time-resolved synchrotron X-ray diffraction (XRD).

2. Experimental Section

2.1. Extrusion-based 3D printing process

A new extrusion-based bioprinting machine, Plasma-assisted Bioextrusion System (PABS), has been developed within the research group ^[38] (**Figure 1**). The PABS system provides a novel approach for biomanufacturing and tissue engineering by combining screw-assisted extrusion, pressure-assisted extrusion, and plasma jetting. PABS not only enables a variety of materials, from cell-laden hydrogels to polymer composites, to be processed but also has the ability to modify the material surface through plasma jetting. This can influence surface properties such as chemical signaling, wettability, surface roughness and energy ^[39-41] which can have an impact on material and biological interactions.

Figure 1. Schematic presentation of screw-assisted extrusion technique

In this study, only the screw-assisted extrusion system was utilized which consists of a material heating chamber and a rotational screw chamber. Materials are deposited in the chamber and the temperature rises above the melting temperature (T_m) to enable material flow. The material is delivered to the screw chamber through the application of compressed air (6 bar) from an externally connected compressor. Heating elements attached to the screw chamber maintain the required temperature, so that the material can be kept molten once delivered to the screw chamber. The rotational screw, driven by a stepper motor (Sanyo Denki,

Japan), is employed to drive the material through the nozzle. A 0.5 mm inner diameter nozzle was utilized in this study forming PCL filaments with a circular cross section.

This extruder presents more precise deposition ability with high melting temperature (up to ~200 °C), faster extrusion speed (up to 20 rpm), improved material mixing, and greater suitability to manipulate materials with high viscosity. The screw rotational speed and melting temperature were kept at 10 rpm and 110 °C. This setting yielded a printed speed of PCL filament at 0.1 mms⁻¹.

2.2. PCL Microstructure

In this study, PCL (CAPA[®]6500, Mw = 50,000 g/mol) purchased from Perstorp (Cheshire, United Kingdom) in the form of 3 mm pellets was used. PCL is a semi-crystalline polymer with a density of 1.1 g/cm³, a melting temperature between 58-60 °C, and a glass transition temperature of -60 °C.

The PCL pellets and printed filaments were sliced using a Leica CM3050 S cryostat to obtain samples of 10 µm and 5 µm thickness respectively, for ex situ optical microscopy. It is important to note that the samples must be thin enough for birefringent spots to be observed as a measurement of the degree of crystallinity, in which the single crystal formula can be applied ^[42]. **Figure 2** shows the microstructures of both as received pellets and filament samples obtained using polarizing light microscope (Olympus BH2-UMA). In the PCL pellet, birefringent spherulitic structures were observed which exhibited the Maltese cross typical of polymers. It is apparent that the size of the spherulites in the PCL pellet were reduced dramatically from a maximum radius of ~40 µm to less than 4 µm for the printed PCL filaments with an increasing number of spherulites, suggesting smaller crystals. *These microscopy images do not allow the observation of any preference in crystal orientation which indicates the importance of in situ investigations through wide angle X-ray diffraction*

to perceive how orientated crystal arrangement evolves during the extrusion process.

Figure 2. Microstructures of (a) PCL raw pellet and (b) extruded filament (melting temperature of 110 °C and screw rotational speed of 10 rpm) obtained using polarized light microscopy.

2.3. In situ synchrotron X-ray diffraction

The evolution of the crystalline structure of PCL during extrusion was examined by using in situ synchrotron wide angle X-ray scattering, which is also referred to as X-ray Diffraction (XRD) at the Diamond Light Source (Harwell, UK) on beamline B16. The beamline's multilayer monochromator delivered X-ray photons with a nominal 17.98 keV ($\Delta E=200$ eV) energy. The beamline's slits were adjusted to a defined 200 x 200 μm^2 beam size for this study. The experimental setup involved simultaneous in situ transmission of XRD and radiographic images (**Figure 3**). However, radiographic imaging was unable to deliver sustained real space information related to the processing of PCL, and consequently is not discussed in this paper. For in situ XRD data collection, a Pilatus[®]300K area detector was mounted at 45° to the beam direction. XRD patterns were collected at 1 Hz frequency (0.9 s exposure time). The distance from the sample to the detector was 266 mm. A boron nitride extrusion nozzle with a 1.5 mm and 0.5 mm external and internal diameter, respectively, was fabricated and used in this research in order to facilitate adequate X-ray penetration. To track

the structural evolution of a pre-selected volume of PCL, the counter motion of the entire print head (horizontally upwards) at 0.1 mm s^{-1} velocity enabled the incident X-ray beam to be illuminated and lock-on to a pre-defined PCL volume that extruded through the printer nozzle. In situ XRD patterns of a moving volume were collected at multiple positions within the nozzle, from a position of just entering the extrusion nozzle to extending 0.1 mm below the nozzle tip. The data position are related to the distance from top to just below the nozzle which are referred to upper most initial position $y = 0 \text{ mm}$ and the subsequent 18 positions increasing with 0.1 mm increments (**Figure 3b**) along the nozzle (where $y = 1.8 \text{ mm}$ is just outside the nozzle) over 18 s duration.

Figure 3. (a) schematic setup of in situ XRD experiment; (b) schematic presentation of nozzle tip and 19 XRD diffraction positions accessed during the experiments (positions: $y=0$ - 1.8 mm).

1 Data processing and background reduction was carried out using a bespoke MATLAB
2 program, which included the conversion of diffraction patterns recorded at 45° angle to the
3 beam direction into observations in the plane orthogonal to the incident X-ray beam
4 employing a rotational matrix for each detector pixel. The pixel intensities were subjected to
5 other standard image/X-ray data processing operations such as radial or azimuthal integrations
6 and peak fitting.
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13 3. Results and Discussion

14 3.1 Crystal orientation

15 Selected in situ X-ray diffraction patterns correspond to positions $y=0-1.8$ mm (time: 0-18 s)
16 are illustrated in **Figure 4a** and in [Movie 1 in the supporting information](#).
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19 The evolution of the PCL XRD patterns reflects the crystal structural changes that PCL
20 undergoes during the printing process (**Figure 4a**) The evolution of low order (020) and (120)
21 reflections from the orthorhombic PCL structure was more prominent in the background
22 corrected images. At the uppermost diffracted positions in the nozzle (ie. positions: $y=0-0.7$
23 mm), a discrete region of each Debye-Scherrer ring was observed for (020) and (120)
24 suggesting crystalline features with a directional alignment. Once the material is pushed
25 through the nozzle (ie. positions: $y=0.8-1.2$ mm), the intensity of the arcs increases while the
26 arc length increases over wider azimuthal angle. At the exit end of the nozzle (ie. positions:
27 $y=1.4-1.8$ mm), the length of the arcs was further increased. Even though the extended arc
28 length of both reflections was visible, initial sections of the arcs appeared brighter compare to
29 the extended regions. The observation indicates the continuation of anisotropic structural
30 preference ^[43], although it has been reduced by some extent when compared to the initial
31 levels.
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Figure 4. (a) Selected in situ XRD patterns from the same volume of PCL as it flows down inside the nozzle during extrusion 3D printing. (b) Refined XRD intensity evolution for (020) at $2\theta \approx 15.3$ and (120) at $2\theta \approx 16.8$ reflections as a function of positions in the nozzle. Lower curve corresponds to position $y=0$ mm, upper curve corresponds to position $y=1.8$ mm.

3.2 Crystal volume fraction

To further elucidate the evolution of crystalline detail during extrusion through the nozzle, the XRD patterns obtained from the 2D area detector (**Figure 4a**) were transformed to a conventional one-dimensional (1D) XRD representation as shown in **Figure 4b**. The transverse axis was recognized as the Bragg angle (2θ). **Figure 4b** shows the scattering

intensity evolution of (020) and (120) reflections for all 18 linear spatial positions as the material flow through the nozzle. The areas under the diffraction peaks are generally proportional to the volume fraction of the crystals ^[44], so that the increasing integrated intensity from position y=0-1.8 mm in the nozzle indicates that the volume of the crystals is increasing during the extrusion of PCL. As shown in **Figure 4b**, the integrated peak intensity increases as the material flows through the nozzle.

Two main characteristics can be identified from the XRD patterns at the top of the nozzle. At position y=0 mm, where the time is taken as 0.0 s, an already crystallized volume can be recognized. This is attributed to the small amount of PCL crystal nucleation taking place before the polymer melt enters the nozzle. Although the temperature was expected to be maintained in both the material heating chamber and screw chamber during the process, as the material flows into the nozzle tip, a temperature gradient is unavoidable between the extruder head and the nozzle. Thus, the PCL melt can be partially undercooled before it reaches the extruder nozzle. It was shown that nucleation and partial crystallization in the PCL were enhanced by substantial undercooling ^[45] as well as the short step stress ^[46]. These are the two potential mechanisms that mostly can be operated in the melt extrusion-based 3D printer. Particularly, the short step stress can be introduced due to the rapid cross sectional change associated when material enters the nozzle from the chamber. The other important feature to be noted is that the crystal orientation was uniform at this stage. [As previously reported the crystallographic c axis of polymer crystals tends to be oriented perpendicular to the extrusion direction, and the preferred crystal orientation \(fiber axis\) is parallel to the drawing direction \(Figure 5\).](#) For further details of crystallographic orientation of polymers readers are referred to ref. [47]. At the beginning arc length is shorter due to crystals organized parallel to extrusion direction, the increased intensity of the initial arc region (azimuthal angles) indicate increased crystal volume fraction oriented in that direction. However, the extension of arc length covering wider azimuthal angle with relatively low intensity at the bottom of the

nozzle suggests a small crystal volume fraction is oriented in a direction deviated from the initial orientation. **Figure 5** shows the evolution of the relative crystal fraction in an increasing manner, but at a gradually reducing rate, along the nozzle distance (relative to the final measurement at $y=1.8$ mm where crystal fraction taken as one). It also presents a schematic presentation of the orientation development during the extrusion of PCL through the nozzle. The results suggest that the shear field generated during printing enhances the formation of oriented crystals in the polymer melts, which is consistent with the literature [48]. The actual flow conditions within the extruder nozzle can be potentially explained by a small boundary layer within the nozzle that introduces a negligible radial velocity profile. However, detailed modelling is currently not available to investigate this hypothesis. Furthermore, the rapid undercooling, with relatively high temperature gradient within the nozzle, appears to be the dominant influencing factor in crystallization in this specific 3D printing extrusion-based process.

Figure 5. Schematic representation of microstructures and crystal volume fraction from top to bottom positions in the nozzle. Crystal volume fraction presents an increasing trend with the material extruding from top to the bottom in the nozzle tip. The majority of the crystal

fraction grows along the axial direction whilst a smaller fraction is observed to grow in other directions with increasing position in the nozzle.

As seen in **Figure 4b**, a slight but gradual shift in 2θ diffraction of (020) and (120) while extruding through the nozzle is typically attributed to thermal contraction. However, considering the reported thermal expansion coefficient of PCL with decreasing temperature along the nozzle, a 2θ change is more than the corresponding thermal shrinkage. The difference can be attributed to the potential compressive strain present in the material. Presence of shear stresses and compressive stress are common phenomena during extrusion processes. Such stress/strain effects must be considered in the future through the use of a precise thermal model for a detailed and quantitative analysis of the extruder nozzle, hence no attempt is made to analyze and present the quantitative figures or stress and stress driven interactions here.

According to the observations, crystallization occurs as the material moves through the nozzle to a substantial level. However, once the material is ejected from the nozzle, further development/evolution of crystallinity is anticipated during subsequent elongation of the polymer because of the printer head stretching the filament during the prescribed x-y movement typical of the 3D printing process. Additionally, as the polymer cools further increase of crystal fraction may occur. Future work includes the investigation of crystallization under different processing parameters and PCL based composites including bioactive ceramics such as hydroxyapatite in order to understand multiscale structural formation in the presence of crystal modifying agents, in addition to the development of quantitative modelling capabilities to simulate the process. This will enable the development of bespoke engineered materials for tissue engineering applications, such as human bone regeneration.

4. Conclusions

1 This contribution provides a unique real time in situ XRD characterization of crystal
2 formation in PCL filaments during melt extrusion-based 3D printing process. The presence of
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4 crystalline anisotropy was identified and the majority of crystals were oriented in the direction
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6 of material flow. In situ time-resolved characterization shows that there is a gradual, but not
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8 complete, reduction of directionality with the increasing crystal fraction of the PCL crystals
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10 during flow through the extrusion nozzle. In addition, experimental and data analysis
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12 procedures presented here demonstrate a novel example and a promising method to employ
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14 real time synchrotron XRD as a tool for probing additive biomanufacturing processes.
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24 **Acknowledgements**

25
26 The authors wish to acknowledge the funding provided by the Engineering and Physical
27
28 Sciences Research Council (EPSRC) and Medical Research Council (MRC) Centre for
29
30 Doctoral Training in Regenerative Medicine (EP/L014904/1). Diamond Light Source is
31
32 acknowledged for granting beamtime (MT14877-1) and authors are grateful to Dr. Kawal
33
34 Sawhney for his advice on experimental design.
35
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39 Technical support from Andrew Malandain (DLS), Kenneth Gyves and Marie O'Brien (UoM)
40
41 is also acknowledged and greatly appreciated.
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44 **Keywords**

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46 Additive biomanufacturing, crystallization, in situ X-ray diffraction, screw-assisted melt
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48 extrusion
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51 **References**

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