THE INFLUENCE OF MULTIPLE THERMAL TREATMENTS OF PCL FILAMENTS INTENDED FOR 3D PRINTING ON THEIR PROPERTIES FOR BIOMEDICAL APPLICATIONS

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Abstract

Multiple heat treatments of polycaprolactone (PCL) during processing cycles can exert a significant influence on both the mechanical and biological properties of filaments intended for 3D printing via Fused Deposition Modeling (FDM). This phenomenon is particularly critical for materials designed for biomedical applications, such as tissue engineering scaffolds, where unintended degradation can lead to a loss of biocompatibility and structural integrity. The primary objective of this research was to systematically evaluate the effects of repeated extrusion on PCL variants with three distinct molecular weights: 25 kDa, 37 kDa, and 50 kDa.

To simulate realistic manufacturing and recycling conditions, the materials underwent a multi-stage extrusion process consisting of three successive cycles. The characterization included measurements of filament diameter consistency, thermal stability via Differential Scanning Calorimetry (DSC), mechanical properties through tensile testing, and comprehensive cell viability assays (XTT) using the Saos-2 cell line, with all results verified by one-way ANOVA and Tukey's post-hoc tests.

The results demonstrated that the impact of repeated heat treatment depends strictly on the molecular weight. PCL 50 kDa exhibited a gradual decline in mechanical and biological properties, suggesting limitations for its extensive reuse in high-precision medical contexts without additional stabilization. Conversely, the PCL 37 kDa variant showed remarkable stability across all cycles, maintaining its structural and functional integrity ($p>0.05$). Furthermore, PCL 25 kDa showed improved cytocompatibility ($p<0.001$), supporting the "thermal cleaning" hypothesis. Overall, PCL 37 kDa emerged as the most reliable grade for sustainable, multi-cycle additive manufacturing in tissue engineering.

Keywords: *PCL, multiple heat treatment, 3D bioprinting, tissue engineering, biomedical engineering*

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Introduction

Tissue engineering is an effective alternative for treating damaged tissues by the creation of three-dimensional scaffolds, which are the place of attachment and the multiplication of cells, often made of polymeric materials [1]. PCL, a semi-crystalline aliphatic polyester, is widely recognized for its biodegradability, biocompatibility, and low melting temperature (~60°C), making it a versatile material for biomedical applications, such as tissue engineering scaffolds and drug delivery systems [2-3]. In PCL processing, temperature is a critical parameter as it significantly influences the material's crystallinity. On the other hand, the kinetics of crystallinity are influenced by the molecular weight of the polycaprolactone [4-6].

One critical aspect of PCL is its melt memory effect, where the polymer retains some crystalline structure even after melting. This phenomenon occurs due to incomplete dissolution of self-nuclei, which influences subsequent crystallization during cooling. Studies have shown that PCL's crystallization rate and spherulitic growth are significantly affected by molecular weight, with repeated heating and cooling cycles exacerbating this effect. Higher molecular weight PCL samples display stronger melt memory, enhancing nucleation density and improving crystallization kinetics. These changes directly affect the mechanical properties of PCL, as more pronounced crystallization during cooling results in improved stiffness and tensile strength [2].

Depending on processing parameters, such as printing speed, nozzle temperature, and flow rate, J. Guerra et al. [7] showed that printed stents using different values of the aforementioned parameters differ in length, thickness, and width of individual segments. The structure of the material also changed, which is related to the change in mechanical properties.

Thermal cycling also impacts the thermal properties of PCL, particularly its crystallization and melting temperatures. Successive thermal treatments can either enhance or degrade PCL's mechanical resilience, depending on the number of cycles and the processing temperatures used. For example, repeated heating to temperatures near or above PCL's melting point (~60°C) can lead to alterations in chain mobility and lamellar thickness, which in turn modify the polymer's crystallinity. This is particularly relevant for applications that involve multiple heat cycles, such as in additive manufacturing or self-healing systems. Repeated heating has been found to increase the rate of crystallization up to a point, after which mechanical degradation may occur. The biological properties of PCL, particularly its biocompatibility and degradation behavior, are also influenced by thermal treatment. PCL undergoes enzymatic or hydrolytic degradation, and thermal treatment can modify its degradation rate by altering the degree of crystallinity [8]. Higher crystallinity generally slows degradation, which is critical in biomedical applications where controlled biodegradation is required. Conversely, excessive thermal treatment may induce chain scission, potentially leading to premature degradation or compromised mechanical integrity. One-time thermal treatment of polycaprolactone can introduce many changes in the material. This phenomenon was the subject of extensive research, but there is still a lack of studies that could answer the question of how multi-heat treatment impacts the mechanical and biological properties of PCL.

However, repeated thermal treatment can have profound effects on its structural and functional characteristics, influencing its suitability for various applications. In summary, while PCL's favorable thermal and mechanical properties make it an attractive material for biomedical applications,

TABLE 1. Comparison of the properties of polycaprolactone with different molecular weights (own elaboration based on [7, 14, 16, 17, 18-22]).

PCL molecular weight [kDa]	10	27	45	50	54	80	100	219
Young Modulus [MPa]	–	2.3[19]	75[21]	180[14]	1.8[19]	400[22]	1.7[19]	1.5[19]
Porosity [%]	20, 69[16]	91[19]	–	42[7]	91[19]	56, 88 [16]	92[19]	94[19]
Compressive Modulus [MPa]	10[18]	–	30[18]	130[17]	–	140[18]	–	–
Viscosity [Pa*s]	90[20]	60[19]	1000[21]	–	800[19]	15000[21]	9000[19]	12000[19]
Sample type	3D printed scaffolds, Electrospun fibers, and HA/PCL composites	Highly porous foam	Solid cast disk	3D printed scaffolds	Highly porous foam	Solid thin film	Highly porous foam	Highly porous foam

repeated thermal treatment presents challenges and opportunities. Optimizing thermal cycles can enhance PCL's crystallization kinetics and mechanical properties, but excessive cycling may result in degradation. The balance between these effects is crucial for applications where repeated processing or long-term stability is required [2, 9].

During polymer evaluation, attention should be paid to the molecular weight, as it can significantly influence the biological and mechanical properties of the material, which is summarized in TABLE 1. It should be noted, however, that the mechanical properties of PCL, particularly the wide range of Young's modulus values reported in literature, are highly dependent on the sample's macro – and microstructure (e.g., induced porosity in scaffolds or foams) rather than solely on molecular weight. Therefore, to accurately investigate the relationship between multiple heat treatments and the molecular weight of polycaprolactone, it is essential to analyze materials across a range of molecular weights under consistent processing conditions.

Therefore, the primary objective of this study was to evaluate the impact of multiple heat treatments on the mechanical and biological properties of polycaprolactone with three different molecular weights (25 kDa, 37 kDa, and 50 kDa), which are commonly employed in biomedical applications. To simulate realistic processing conditions and assess the feasibility of material recovery and reuse, the PCL variants were subjected to three successive extrusion cycles.

Materials and methods

The starting materials for this study were three grades of polycaprolactone (PCL) in pellet form, purchased from Polysciences, Inc., with molecular weights of 25 kDa, 37 kDa, and 50 kDa.

Samples extrusion

The filament extrusion process was performed using a 3Devo Composer 450 extruder, equipped with four independent heating zones (FIG. 1). To ensure optimal dimensional accuracy and to account for the varying melt viscosities associated with the different molecular weights, specific temperature profiles were tailored for each PCL variant and extrusion cycle.

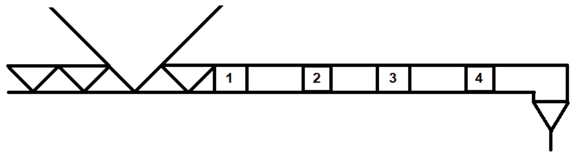

FIG. 1. Diagram of the heater arrangement in the 3Devo Composer 450 filament extruder.

The specific extrusion parameters for each PCL variant and processing cycle are summarized in TABLE 2. The heating zones (Heaters 1-4, as illustrated in FIG. 1) were maintained at temperatures near the melting point of PCL to prevent excessive thermal degradation while ensuring sufficient melt flow. The extruder speed was kept relatively constant at approximately 2.0 RPM to maintain a stable filament diameter of 1.75 mm. For the third cycle of the 25 kDa variant (W_III), the speed was adjusted manually to compensate for the altered rheological behavior of the recycled material.

Multi-stage extrusion and material recovery

The extrusion process, described below, was the same for every PCL molecular weight.

Extrusion I (W_I):
- extruding a filament from 300 ml of starting material (pellets),
- cutting the filament that was extruded from the starting material into pellets with a segment length of ≤ 4 mm.

Extrusion II (W_II):
- extruding the filament from 200 ml of material cut into pellets after the first extrusion (W_I),
- cutting the filament from W_II into pellets with a segment length of ≤ 4 mm,

Extrusion III (W_III):
- extruding the filament from 100 ml of material cut into pellets after the second extrusion (W_II).

TABLE 2. Detailed extrusion parameters for PCL filaments across different molecular weights and processing cycles.

Parameters/PCL [kDa]	25 W_I	25 W_II	25 W_III	37 W_I	37 W_II	37 W_III	50 W_I	50 W_II	50 W_III
Heater 1 [°C]	64	62	67	66	65	65	68	68	69
Heater 2 [°C]	66	66	66	68	67	65	66	66	67
Heater 3 [°C]	68	68	68	69	68	67	68	68	70
Heater 4 [°C]	59	59	59	61	60	60	59	59	59
Extruder speed [RPM]	1.9 ÷ 2.1		variable	1.9 ÷ 2.1			1.9 ÷ 2.1		
Cooling temperature [°C]	21								
Filament diameter [mm]	1.75								

Measurement of the accuracy of maintaining the diameter dimension of the extruded filament

For the initial filament extrusion (W_I) of PCL 25 kDa, 37 kDa, and 50 kDa, three samples were prepared to evaluate the dimensional accuracy of the produced 3D printing material. Dimensional accuracy was assessed using a digital vernier caliper at five distinct points. From the obtained results, an average was drawn for each sample.

Determination of melting point by differential scanning calorimetry (DSC)

Melting points were determined using differential scanning calorimetry by performing measurements on a Netzsch's DSC 204 F1 Phoenix instrument. For the study, three samples each were prepared for pellets, W_I, W_II and W_III. The test samples were heated in the temperature range from - 60°C to 100°C using liquid nitrogen to cool them down. The test was carried out in three cycles: heating-cooling (with liquid nitrogen)-heating.

Young's modulus of extruded filaments

The Young's modulus of the extruded filaments was determined through tensile strength tests conducted on a Bruker tribotester (UMT-2). Given the specific nature of 3D printing materials, measurements were performed directly on cylindrical filament samples secured in the grips of the testing machine. Each sample had a total length of 40 mm and a nominal diameter of 1.75 mm. This approach was selected to characterize the material in its ready-to-use form for the FDM process. To ensure statistical reliability, ten samples were tested for each PCL variant (25 kDa, 37 kDa, and 50 kDa) at every extrusion stage (W_I, W_II, and W_III). The tests were conducted at a constant speed of 5 mm/min. Young's modulus was calculated from the linear slope of the stress-strain curves.

Due to the filament form of the samples, specialized grips were designed and utilized to ensure proper and reproducible mounting in the testing machine (Figure 2). This custom clamping system was specifically engineered to prevent slippage during tensile testing without damaging the delicate PCL structure.

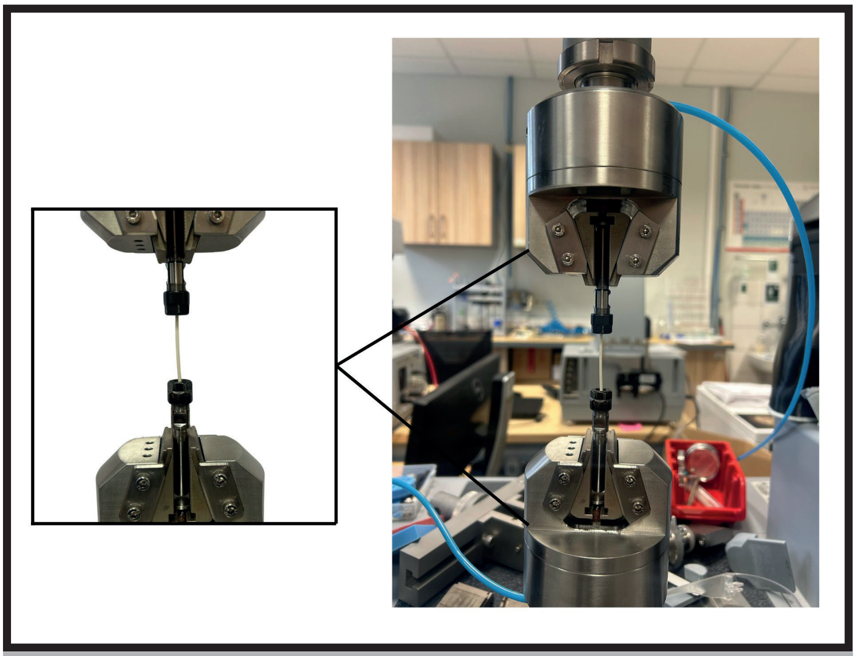

FIG. 2. Detailed view of the custom grip assembly designed to maintain axial alignment and secure fixation of the PCL "string" samples during tensile strength tests.

Test of cell viability in the XTT test with the use of extracts from extruded filaments

The XTT test was carried out on the extracts made of the tested samples, which were prepared according to the ISO 10993-12 standard. First, samples of filaments with 25 kDa, 37 kDa, and 50 kDa PCL from the extrusion W_I, W_II, W_III, and the starting material P (pellets) were placed in the Eppendorf tubes and then immersed in the culture medium (McCoy's + 15% FBS + 1% P / S). The samples prepared in this way were incubated for 24 h at 37°C. Simultaneously, in 96-well culture plates, osteoblast-like cells of the Saos-2 line were placed at 1x104 cells / well and supplemented with culture medium (McCoy's + 15% FBS + 1% P / S) up to 100 µl.

In this study, two controls were applied – the positive one (cells contacted with 7µl DMSO / well) and a negative one (cells without contact with any extract). Before adding the extracts, the cells were incubated for 24 h in an atmosphere of 5% CO_2, 90% humidity at 37°C. After this time, the medium was removed, and then the previously prepared extracts from PCL samples were added in a volume of 100 µl / well. Cells were incubated with extracts for 24 h, in an atmosphere of 5% CO_2, 90% humidity, and 37°C. After this incubation time, the XTT reagent was added to each well, followed by incubation for a further 4 h. Finally, the absorbance was measured at 450 nm and 660 nm using the PerkinElmer Victor X microplate reader.

Five repetitions were made for each PCL sample with a molecular weight of 25 kDa, 37 kDa, and 50 kDa from extrusions W_I, W_II, W_III, and the starting material. The results were obtained by applying the following formula:

$$Viability[\%] = \frac{A_{450} - A_{660}}{KN_{450} - KN_{660}} * 100\%$$

A_{450} – the absorbance measured at 450 nanometers, which is used to estimate the number of viable cells

A_{660} – the absorbance measured at 660 nanometers, which is the background absorbance

KN_{450} – the absorbance measured at 450 nanometers, which is used to estimate the number of viable cells for the negative control

KN_{660} – the absorbance measured at 660 nanometers, which is the background absorbance for the negative control.

Statistical analysis

The statistical significance of the differences between the experimental groups was evaluated using a one-way analysis of variance (ANOVA). For all parameters where ANOVA indicated significant differences ($p < 0.05$), a post-hoc Tukey's Honest Significant Difference (HSD) test was performed to conduct pairwise comparisons. In the biological evaluation (XTT assay), the results were compared against the raw pellet control to identify changes in cytocompatibility across all processing stages (W_I, W_II, W_III). For mechanical tests, the comparisons focused on the stability across consecutive extrusion cycles (W_I vs W_II vs W_III). For dimensional accuracy, the analysis was performed to identify significant differences between the three PCL molecular weight variants during the initial filament formation stage (W_I). All statistical calculations were performed using the StatsKingdom statistical software toolkit, and the results are presented as mean values ± standard deviation (SD). The significance levels were set at * $p < 0.05$, ** $p < 0.01$, and *** $p < 0.001$.

Results

The analysis of filament diameters was performed for the initial extrusion stage (W_I) to ensure the material meets the dimensional requirements for standard FDM 3D printing (FIG. 3). All molecular weight variants successfully produced filaments with diameters near the nominal 1.75 mm. PCL 25 kDa and PCL 37 kDa exhibited high dimensional consistency, with average diameters of approximately 1.76 mm and 1.69 mm, respectively. In contrast, the PCL 50 kDa variant showed a higher initial variance and a larger average diameter (2.22 mm) during the first extrusion cycle.

Filaments produced from PCL 37 kDa exhibited slightly higher melting points (T_m) compared to the 25 kDa and 50 kDa variants (FIG. 4). For all molecular weights, a marginal increase in T_m was observed after the first extrusion (W_I) relative to the starting material (pellet), followed by stabilization in subsequent processing cycles. Statistical analysis of the melting temperatures confirmed the high thermal stability of all PCL grades. For PCL 37 kDa, although a slight temperature rise was noted after the first cycle, the overall variation was found to be statistically non-significant ($p = 0.982$). Similarly, for PCL 25 kDa ($p = 0.186$) and PCL 50 kDa ($p = 0.902$), the fluctuations in melting points were confirmed to be non-significant.

These results, combined with the stable degree of crystallinity (X_c), demonstrate that the primary crystalline phases of all three PCL variants remain remarkably resilient to repetitive thermo-mechanical processing, maintaining their fundamental thermal characteristics.

As shown in FIG. 5, the highest average Young's modulus was recorded for the PCL 25 kDa W_III sample. For the 25 kDa variant, a progressive increase in stiffness was observed in the W_II and W_III cycles compared to W_I, with values eventually surpassing those of the PCL 50 kDa samples from the same stages. This upward trend for PCL 25 kDa was confirmed to be highly significant by one-way ANOVA ($p < 0.001$), with Tukey's post-hoc test showing significant differences for both W_II and W_III vs W_I ($p < 0.001$). In contrast, PCL 37 kDa showed no significant differences across the cycles ($p = 0.989$), confirming its exceptional mechanical stability.

For PCL 50 kDa, the Young's modulus decreased with each successive extrusion. While a clear downward trend in mechanical integrity was observed, the reduction did not reach statistical significance ($p = 0.892$) due to the higher variance in the processed samples. Nevertheless, the results support the conclusion that higher molecular weight PCL is more susceptible to thermo-mechanical chain scission, leading to a loss of structural consistency during repeated recycling.

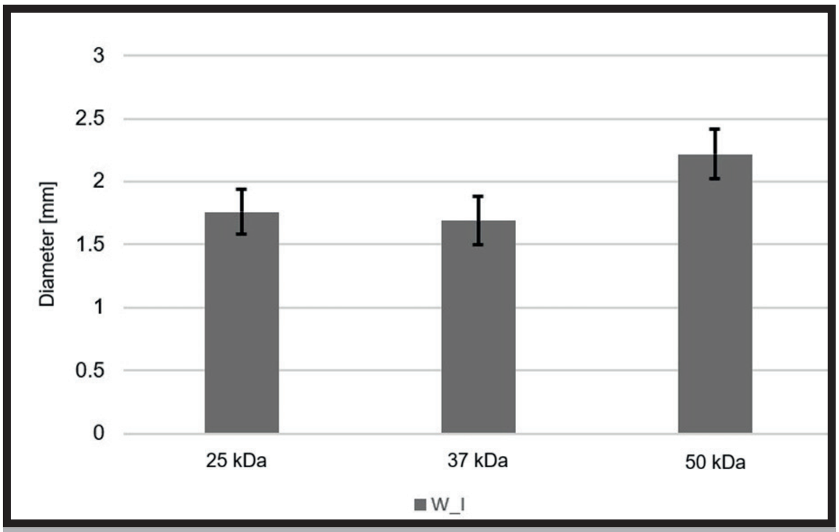

FIG. 3. Results of the PCL filaments diameter measurements for the initial extrusion stage (W_I) for PCL 25 kDa, 37 kDa, and 50 kDa. Diameter measurements data are presented as mean ± standard deviation (n=3 samples prepared for each grade).

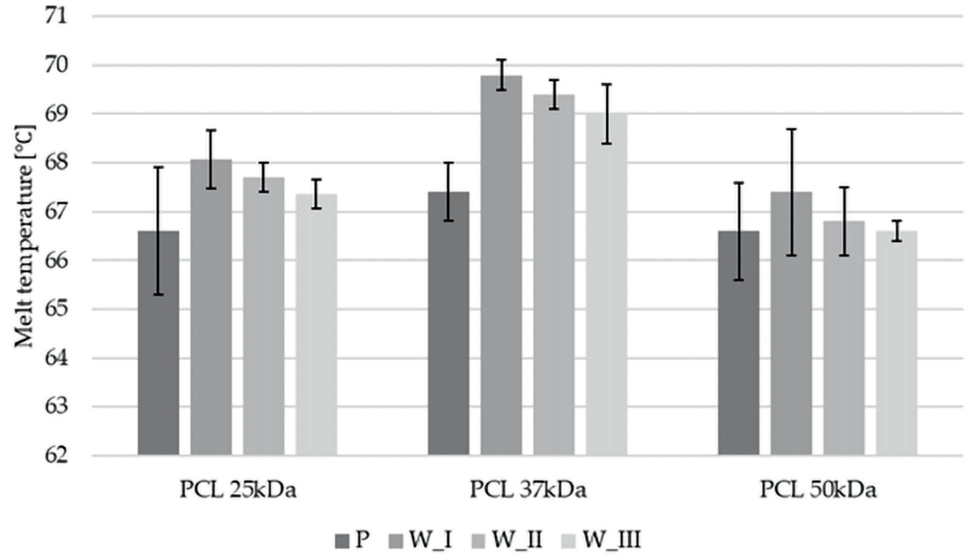

FIG. 4. Results of melting points measurements using DSC test for PCL 25 kDa, 37 kDa and 50 kDa after subsequent extrusions (heat treatment W_I, W_II, W_III). DSC measurements data are presented as mean ± standard deviation (For the study, n=3 samples were prepared for pellets, W_I, W_II and W_III).

FIG. 6 shows the results of the cytotoxicity evaluation using the XTT assay. The biological response varied significantly across the PCL grades. The highest cell viability was recorded for PCL 37 kDa, reaching approximately 118% of the negative control, with all samples of this material maintaining values well above the 80% threshold.

For PCL 25 kDa, a highly significant improvement in cell viability was observed following repeated extrusion cycles ($p < 0.001$). Post-hoc Tukey HSD analysis confirmed that the metabolic activity increased significantly starting from the first cycle (W_I) compared to the raw pellet ($p = 0.021$), peaking at the third cycle (W_III, $p < 0.001$ vs Pellet). This upward trend supports the "thermal cleaning" hypothesis.

In contrast, PCL 37 kDa maintained exceptional biological stability throughout all cycles, with no significant differences observed between the processed filaments and the raw pellet ($p = 0.924$). For PCL 50 kDa, a slight downward trend in viability was noted from W_I (88%) to W_III (73%); however, this decline did not reach statistical significance ($p = 0.351$). Importantly, even after three extrusion cycles, all PCL variants remained non-cytotoxic, supporting the safety of the recycling process for medical-grade polymers.

Discussion

The observed differences in the mechanical, thermal, and biological properties of PCL after successive thermal treatment cycles can be attributed to the effect of cooling rate during extrusion, which directly influences the crystalline

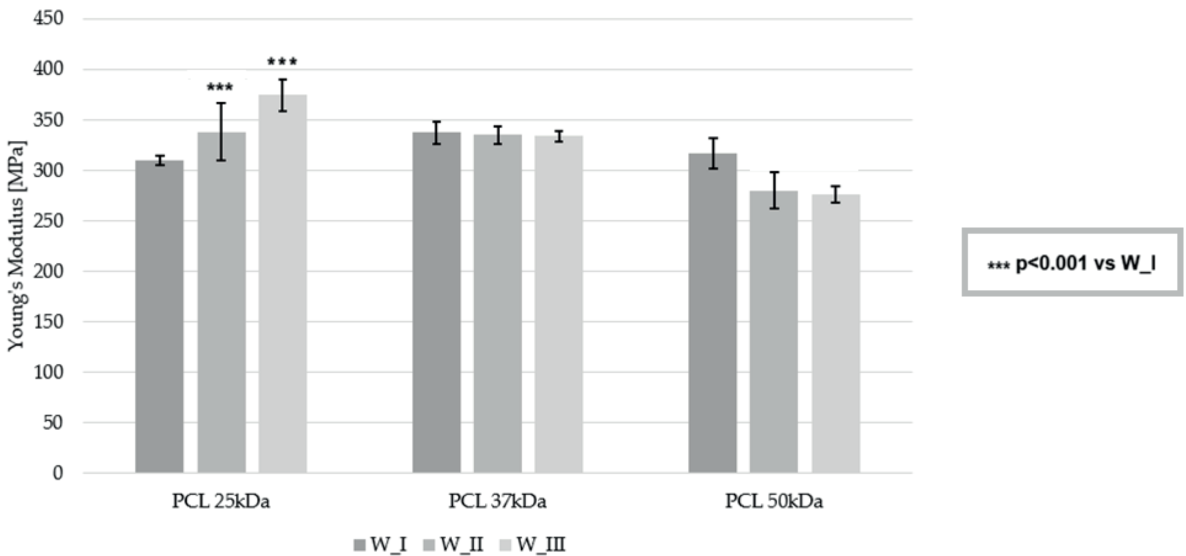

FIG. 5. Results of Young's Modulus measurements for PCL 25 kDa, 37 kDa and 50 kDa and after subsequent extrusions-heat treatment W_I, W_II, W_III (Young's Modulus data are presented as mean ± standard deviation for n=10 longitudinal samples were prepared for each PCL extrusion with molecular weights of 25 kDa, 37 kDa and 50 kDa).

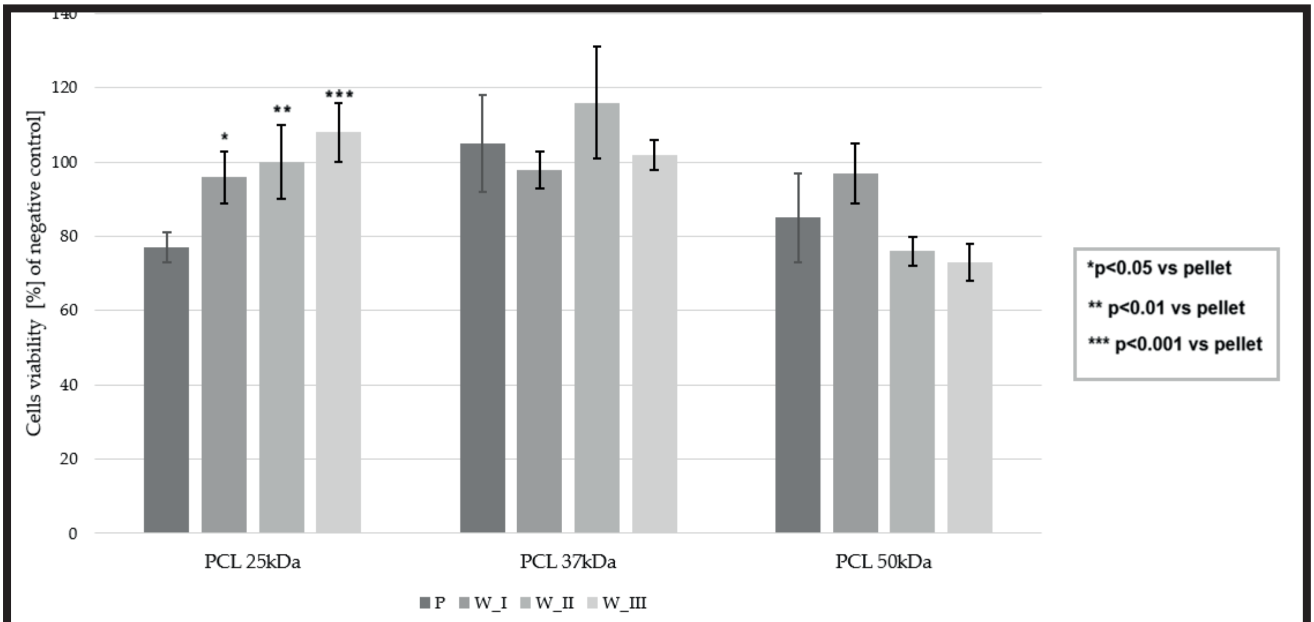

FIG. 6. Results of cell viability measurement by XTT test for PCL 25 kDa, 37 kDa and 50 kDa in form of pellet (P) and after subsequent extrusions-heat treatment W_I, W_II, W_III (Cell viability data are presented as mean ± standard deviation from n=5 repetitions were made for each PCL sample with a molecular weight of 25 kDa, 37 kDa and 50 kDa from extrusions W_I, W_II, W_III and the starting material.)

structure of the material. A key factor determining the cooling rate and its consequences is the molecular weight of the polymer, which governs both the viscosity of the molten material and the crystallization kinetics.

Filament diameter consistency is a critical requirement during the initial production phase (W_I) to ensure reliable material feeding during the FDM process. For the 50 kDa variant, the high melt viscosity led to less stable flow during this initial stage, resulting in higher variance and a larger average diameter compared to the lower molecular weight variants. However, as the subsequent processing cycles simulate the 3D printing and recycling stages where the material is reshaped by the printing nozzle, the initial filament geometry becomes less relevant to the final scaffold's structural properties.

Thermal analysis via Differential Scanning Calorimetry (DSC) revealed significant shifts in the melting behavior of PCL across successive processing cycles. The observed increase in melting temperature (Tm) after the first extrusion cycle (W_I) for all molecular weights suggests an improvement in the crystalline organizational state. This phenomenon can be attributed to the reorganization of polymer chains into more perfect and thicker lamellar structures during the controlled melt-recrystallization process, which effectively alleviates the internal stresses present in the raw material. Although the recorded shifts in melting temperatures between cycles were subtle (approx. 1°C), they represent a consistent trend indicative of gradual polymer degradation. These findings align with the observed decline in mechanical stiffness and biological response, particularly for higher molecular weight PCL, confirming that even minor thermal changes can signal a reduction in the material's long-term stability. Regarding the stability of the crystalline phase, our study demonstrates that the recorded shifts in melting temperatures remained statistically non-significant for all grades, including the 37 kDa variant ($p = 0.982$). This confirms that while the extrusion process acts as a controlled annealing step (improving order from Pellet to W_I), the bulk crystalline structure remains resilient to further thermal stress during recycling.

This confirms that the DSC measurements are sensitive enough to detect structural changes. However, the lack of significant T_m shifts in PCL 50 kDa, despite its mechanical failure, suggests that for high-molecular-weight PCL, functional degradation (loss of stiffness and biocompatibility) precedes any major changes in the bulk crystalline melting behavior. The subsequent decline in Tm during additional processing cycles (W_II and W_III) points toward a gradual reduction in the degree of crystallinity and lamellar perfection. This trend is likely driven by cumulative thermo-mechanical degradation, specifically chain scission, which reduces the average molecular weight and increases polydispersity. Shorter, degraded chains exhibit a diminished capacity to form large, stable crystalline domains, resulting in a shift of the endothermic melting peak toward lower temperatures.

Furthermore, the raw pellets (P) exhibited the lowest melting temperatures across all groups. This can be explained by their 'industrial thermal history'-typically involving rapid cooling or injection molding during mass production, which leads to a kinetically trapped, less-ordered crystalline morphology. The first thermal treatment (W_I) essentially acts as a controlled annealing step, allowing the material to overcome these initial structural irregularities and develop a more thermodynamically stable crystalline phase. These findings are consistent with the results reported by Jenkins and Harrison (2006), who demonstrated that PCL often possesses a lower degree of crystalline order compared to processed samples, thereby requiring less energy for the transition to a disordered melt state [12].

Mechanical characterization revealed a progressive increase in the Young's modulus for PCL 25 kDa following each successive extrusion cycle. The error bars for Young's modulus reflect the inherent structural differences between individual filament segments. The narrowest distribution of results for PCL 37 kDa suggests a more uniform crystalline structure across the samples compared to the 25 kDa and 50 kDa variants. This trend can be attributed to the high molecular mobility of shorter polymer chains, which facilitates the nucleation and growth of a higher density of small, uniform crystallites during the rapid cooling phase of the extrusion process. These finely dispersed crystalline regions act as reinforcing sites within the amorphous matrix, enhancing mechanical interlocking and macroscale stiffness. This observation aligns with the literature suggesting that lower molecular weight PCL solidifies more efficiently, promoting a more ordered crystalline fraction that strengthens the bulk material [2, 6].

In contrast, PCL 37 kDa demonstrated remarkable structural stability, maintaining a consistent elastic modulus across all reprocessing stages (W_I–W_III). This stability indicates that PCL 37 kDa occupies an optimal processing window, where the material maintains a delicate balance between its amorphous and crystalline phases despite repeated thermal history. Unlike the 25 kDa and 50 kDa variants, which exhibited opposing trends in stiffness, the 37 kDa variant appears to combine the benefits of sufficient chain length with resistance to significant morphological alterations [8].

Conversely, the 50 kDa variant exhibited a gradual decline in Young's modulus with each extrusion cycle. While this downward trend correlates with potential thermo-mechanical chain scission, it did not reach statistical significance ($p = 0.892$) in this study, likely due to the increased variability in the degraded polymer segments. For high molecular weight polymers, multiple thermal treatment cycles often induce thermo-mechanical degradation and chain scission. The resulting reduction in effective chain length and the potential increase in polydispersity likely disrupted the continuity of the polymer network and hindered the formation of a stable crystalline phase, leading to the observed loss of mechanical integrity. These findings suggest that while PCL 37 kDa provides the highest process stability for recycled 3D printing filaments, PCL 50 kDa is significantly more susceptible to degradation-induced softening during multiple extrusion cycles [2, 23].

The consistent modulus of elasticity also suggests that even after multiple processing cycles, the material maintains a stable balance between its crystalline and amorphous phases without significant morphological alterations. A similar phenomenon was described by Liu et al. (2019), who indicated that controlled recrystallization and moderate molecular weight support the retention of favorable mechanical properties even after several reprocessing cycles [8, 9, 14]. The lower Young's modulus values compared to the theoretical maximum (440 MPa) are likely due to the process-induced semi-crystalline morphology. The rapid cooling during extrusion inhibits full crystal growth, as supported by the DSC results. While the use of standardized samples (e.g., ISO 527) is preferred for a direct comparison with general bulk material literature, evaluating the filament 'as-is' provides valuable information on the direct impact of multi-stage extrusion on the raw 3D printing material's properties. The custom grips ensure the reliability of these in-situ tensile measurements.

The biological evaluation using the XTT assay on the Saos-2 osteoblast-like cell line provided critical insights into the safety of reprocessed PCL. Cell viability for all samples and in each form was above 70% compared to the control, which, in accordance with the guidelines of the ISO 10993-12 standard, proves the lack of cytotoxicity for the tested materials. Regardless of this, it should be noted that PCL 37 kDa exhibited the highest cell viability, reaching approximately 118% of the negative control, with all processing stages (W_I–W_III) maintaining viability levels well above the 80% threshold. This suggests that the moderate molecular weight of 37 kDa PCL provides sufficient thermal stability to prevent the significant leaching of toxic degradation products during multiple extrusion cycles.

In contrast, PCL 50 kDa showed a clear downward trend in cytocompatibility. While W_I showed high viability, the value for W_III dropped to the 70% limit. While this downward trend correlates with the thermo-mechanical degradation observed in other tests, it is important to note that the viability remained within the non-cytotoxic range (above 70%) according to ISO 10993-5. Cumulative thermal stress in high MW PCL can lead to the formation of acidic by-products, such as 6-hydroxycaproic acid, which may lower the local pH of the culture medium and negatively impact cell metabolic activity [7]. The use of the XTT metabolic assay, conducted according to ISO 10993-5, allowed for a precise quantitative assessment of the metabolic health of Saos-2 cells in contact with PCL extracts. This approach is particularly effective in detecting subtle shifts in cytotoxicity caused by potential thermal degradation products, such as 6-hydroxycaproic acid, providing a clear threshold for the safe reuse of the polymer.

Interestingly, PCL 25 kDa pellets showed an initial viability below 80%, which then increased after the first and second extrusion cycles. This improvement might be attributed to a "thermal cleaning" effect, where the initial extrusion process aids in the removal or stabilization of residual monomers or industrial additives present in the raw pellets. It is also worth bearing in mind that the pellet form provides a larger surface area of the material for the possible active release of cell-sensitizing compounds into the extract, which could also have influenced the observed biological response. These preliminary biological findings provide a baseline for future long-term biocompatibility and cell-scaffold interaction studies. Overall, these above described results confirm that PCL 37 kDa is the most biostable grade for multiple-cycle additive manufacturing [24, 25].

Conclusions

Based on the experimental results and the subsequent analysis of the thermal, mechanical, and biological properties of reprocessed polycaprolactone, the following conclusions can be drawn:

1. The molecular weight (MW) of PCL is the critical factor governing the material's response to multiple thermal treatment cycles. It dictates melt viscosity, cooling kinetics, and the resulting crystalline morphology, which collectively determine the final mechanical integrity and biological safety of the 3D-printed constructs.
2. Low molecular weight PCL (25 kDa) exhibits a unique stiffening effect characterized by an increase in Young's modulus after successive extrusion cycles. This is attributed to high chain mobility facilitating the formation of a dense network of small, uniform crystallites. While biologically stable, this grade requires rigorous dimensional control due to its low melt viscosity.
3. Among the tested variants, PCL 37 kDa demonstrated the highest structural and mechanical stability. Its moderate MW provides an optimal processing window, maintaining a consistent elastic modulus and superior cell viability (Saos-2) throughout multiple cycles. Consequently, 37kDa is recommended as the most reliable grade for reproducible manufacturing in tissue engineering.
4. High molecular weight PCL (50 kDa) is highly susceptible to cumulative thermo-mechanical degradation (chain scission) during reprocessing. This results in a gradual reduction in Young's modulus and a downward trend in biocompatibility. Consequently, it is recommended to exercise caution when reusing this specific grade in biomedical applications, as it may require more rigorous quality control to ensure structural and biological safety.

5. The initial extrusion cycle (W_I) acts as a controlled annealing step that improves structural order and increases the melting temperature (Tm) relative to raw pellets.
6. Comprehensive statistical analysis using one-way ANOVA and Tukey's HSD post-hoc test confirmed that multiple processing cycles significantly enhance the mechanical stiffness and cytocompatibility of PCL 25 kDa ($p < 0.001$), supporting the 'thermal cleaning' effect. In contrast, PCL 37 kDa was identified as the most structurally and dimensionally stable variant ($p > 0.05$ for all parameters), while PCL 50 kDa showed higher initial variance during filament formation, indicating challenges in its initial processability compared to lower molecular weight variants.

However, subsequent cycles (W_II, W_III) induce progressive degradation and disrupt the crystallization process, leading to a systematic decline in (Tm) across all molecular weights. In summary, the sustainable use of PCL in additive manufacturing requires a strategic selection of molecular weight. PCL 37 kDa represents the optimal compromise between mechanical performance, thermal resistance, and processability, making it the ideal candidate for advanced bioprinting and material recovery protocols.

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