THE EFFECTS OF B-IRRADIATION ON ELECTROSPUN POLYCAPROLACTONE FIBRES

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https://doi.org/10.37904/nanocon.2024.5027

Abstract

Microplastics originating from the degradation of plastic waste are considered a global concern for the environment, with microplastic fibres from synthetic textiles being responsible for the majority of microplastic pollution. Over the past few decades, there has also been a notable increase in the production of synthetic polymeric materials in the form of nanofibers for various applications. Despite this fact, knowledge in the field of aging of nanofibrous materials is very limited. To evaluate the effects of polymer aging, accelerated aging techniques involving high-energy irradiation are commonly used. This work focuses on the impact of β-irradiation on electrospun polycaprolactone nanofibers. Polycaprolactone (PCL) is a semicrystalline biodegradable polyester widely used in medical applications; thus, any potentially formed fiber fragments pose less risk to the environment and health compared to non-biodegradable counterparts. The impact of irradiation was evaluated in terms of the mechanical properties of the prepared nanofibrous mats. Additionally, the effects on fibre morphology and crystallinity were assessed, as well as the impact of irradiation on the biocompatibility of the material.

Keywords: β-irradiation, polymer aging, polycaprolactone, nanofibers

1. INTRODUCTION

Plastics or in another words synthetic polymers found their way to variety of applications since the discovery of first synthetic polymer bakelite in 1909. Wide spread of plastics was boosted due to its unique mechanical and chemical properties, as well as low production costs in comparison to conventional materials [1]. Worldwide production of plastics is still increasing to this day, which goes in hand with production of plastic waste. Around 80 % of plastic waste ends up in landfills or in the environment, thus significantly changing the biosphere [1, 2]. Furthermore, plastic waste in the environment is subjected to numerous forces, which facilitate disintegration into smaller particles, eventually generating secondary microplastics (< 5 mm) and nanoplastics (< 100 nm) [2]. Vast majority of microplastic pollution in aquatic ecosystems is in form of microplastic fibres originating from synthetic textiles [3]. In addition to conventional synthetic microfibrous materials, nanofibers found its way to multiple applications in last decades, which may end up in environment in some form of waste after successfully fulfilling its lifecycle [4]. Despite this fact there is little to no information available about fate of nanofibrous materials, when subjected to environment.

Aging of polymers is understood as a change of mechanical and chemical properties over the time [5]. Polymer aging is complex process influenced by many factors – sunlight exposure, temperature, humidity, external stress, to highlight few [6]. In case of polymers with C-C backbone aging process is initiated by UV irradiation, which causes breakage of C-H bonds within polymeric chain forming free radicals, followed by formation of peroxy radicals in presence of oxygen. Subsequent radical reaction result in further chain scission, crosslinking, branching or formation of oxygen-containing groups. For polyesters UV irradiation results in ester bond cleavage, as well as formation of carboxyl and hydroxyl groups, which further promote hydrolysis of ester bonds in aqueous environment. Thus photo-oxidation results mainly in chain scission in case of polyesters [7].

Standard approaches to study aging process of polymeric materials employ accelerated aging methods. Accelerated aging methods were originally developed to predict lifecycle and time depend performance of polymeric materials within certain application, which is otherwise extremely time-consuming. Since solar irradiation is considered main reason of polymer aging, majority of commonly used accelerated aging methods is based on UV irradiation [8]. Despite this fact, proposed study suggests use of high energy electrons (β-irradiation) to study aging of polymeric nanofibers, which allows even further reduction in time-consuming nature of the experiment in comparison to UV irradiation.

2. MATERIALS AND METHODS

Materials

Polycaprolactone PURASORB® PC 08 was obtained from Corbion (Netherlands), chloroform and ethanol were purchased from Penta (Czechia). PCL in form of pellets was dissolved in chloroform/ethanol mixture in ratio 8:2 by weight resulting in 16% PCL solution (w/w). The solution was stirred over night at room temperature. Nanofibrous layers were prepared by needless electrospinning device Nanospide™ NS 1WS500U (Elmarco, Czechia) equipped with 200 µm string as spinning electrode using following parameters: spinning electrode voltage of 40 kV, collector voltage of -10 kV, spinning electrode – collector distance of 180 mm, polymeric solution dosing device with aperture of 700 µm, substrate winding speed of 33 mm/min, temperature of 22 °C and relative humidity of 50 % inside the spinning chamber. Formed nanofibers were deposited on polypropylene nonwoven substrate, for purpose of following experiments nanofibrous layer was separated from supporting substrate. Final grammage of PCL nanofibrous layer obtained by needless electrospinning was 33 g/m^2.

Radiation resistance testing

Electrospun nanofibrous layers were cut into rectangles (70 × 100 mm) and sealed in sterilization packaging material. Afterwards 45 samples were placed into custom made frame to ensure homogenous irradiation of all samples. Sample irradiation was conducted with microtron MT25 located at Nuclear Physics Institute, CAS in Prague. The electron beam with intensity of 16,5 MeV and dose rate of 1,56 Gy/s was used for sample irradiation. Frame with samples was placed on rotating base at distance of 450 mm from microtron outlet, rotation around vertical axis ensured homogenous irradiation of all samples in the frame. The aluminium foil (thickness of 3 mm) was placed at distance of 120 mm from microtron outlet to disperse electron beam, resulting in homogenous field 100 × 100 mm in the target plane allowing for irradiation of entire area of nanofibrous samples. During the irradiation, samples were cooled by stream of cold air to prevent overheating caused by electrons colliding with material. Individual samples were removed from the frame after obtaining desired dose. Experimental setup is depicted in **Figure 1**.

Morphological and structural analysis

The morphology was analysed using scanning electron microscopy (SEM) Vega 3, Tescan, Czechia). Samples for SEM analysis were coated by gold to ensure surface conductivity using sputter coater Q150R ES (Quorum,

United Kingdom). Fibre diameter was measured by image analysis software ImageJ, average fibre diameter for each sample was determined as average value of 250 measurements from 5 SEM images. The impact of β-irradiation on internal structure of PCL nanofibers was analysed by differential scanning calorimetry (DSC), (DSC 1/700, Mettler Toledo, Switzerland). For each sample 2 measurements were conducted, samples were heated from -20 to 100 °C at a heating rate of 10 °C/min.

Tensile testing

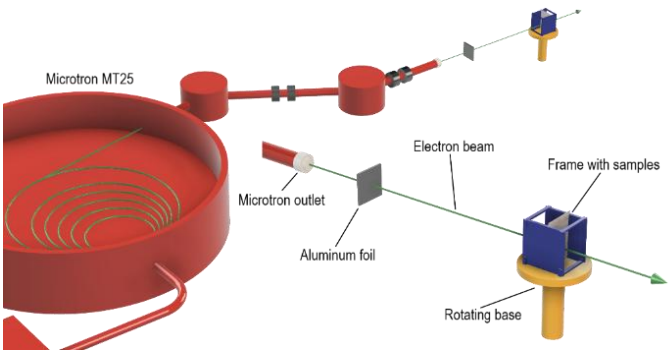
For tensile testing irradiated and control samples were cut into strips with width of 10 mm. Obtained samples were afterwards attached to paper supporting frame to ensure parallel axial alignment in tensile tester grips. Subsequently supporting paper frame was cut before measurement was performed. Tensile tests were carried out by LabTest 6.0051 (LaborTech, Czechia) universal tensile tester equipped with 10 N (accuracy class: 1 from 0,030 N according to EN ISO 7500-1, accuracy class: 0,5 from 0,1 N according to EN ISO 7500-1) load cell, (AST, Germany), with sampling frequency of 50 Hz. For all samples, tensile tests were performed with initial distance of 20 mm and velocity of 10 mm/min, preload of 0.1 N. Average values of mechanical properties were determined as average value of 10 measurements.

Cytotoxicity testing

Cytotoxicity testing was conducted following EN ISO 10993-5 (855220) with 3T3 mouse fibroblasts. DMEM High Glucose (Dulbecco's Modified Engles Medium, Merck) with 10% Fetal Bovine Serum (Biosera, Czechia), 1% glutamine (Biosera, Czechia) and 1% antibiotic - Pen / Strep Amphotericin B (Lonza, Switzerland) was used as a cell culture media. From each sample, circle with a diameter of 14 mm was cut out of sample by die and placed in 24 well microtiter plate with 1 ml of media. Samples were incubated with cells for 24 hours in a CO2 incubator at 37°C (Heracell Vios 160i, Thermo Fisher Scientific, USA). For each sample 8 +2 (control) wells were seeded with cells at cell density of $1*10^4$ cells/ml in DMEM. As a negative control DMEM culture media was used, as positive control served 0.5% wt. of triton in DMEM. Seeded cells were incubated in media for 24 hours, afterwards culture media was extracted and 100 µl of media incubated with tested nanofibrous samples was added to seeded cells. After next 24hour incubation cycle cytotoxicity was evaluated by CCK metabolic test (spectrophotometry analysis).

Statistical evaluation

The statistic evaluation of experiments was performed in MATLAB R2024a software, normality of data was tested by Kolomogorov-Smirnov test, multiple comparisons between groups were performed by Kruskal-Wallis test followed by post hoc Dunnett's test.



Dose [kGy]	*Q [mC]*	*Irradiation time [s]*		*Ḋ [Gy/min]*
50	16 025	T_1	785	3822
100	32 050	T_1+595	1 380	4348
150	48 075	T_2+591	1 971	4566
200	64 100	T_3+589	2 560	4688
250	80 125	T_4+608	3168	4735
300	96 150	T_5+627	3 795	4743
350	112 175	T_6+569	4 364	4812
400	128 200	T_7+577	4 941	4857

Figure 1 Scheme of experimental setup of β-irradiation process of PCL nanofibers. Table representing irradiation times necessary for each sample (dose) and corresponding dose rates *Ḋ*.

3. RESULTS AND DISCUSSIONS

Mechanical properties

Mechanical properties were evaluated by uniaxial tensile test on samples (0–200 kGy). Samples subjected to higher β-irradiation doses (250–400 kGy) were excluded due to severe mechanical degradation, which disallowed attachment of the samples into the testing apparatus. Obtained stress-strain curves are plotted in **Figure 2**. Statistically insignificant differences were observed between sample subjected to dose of 50 kGy and control sample. However, greater doses of resulted in significant decreases in both ultimate tensile strength (up to 70 %), and strain at failure (up to 91 %), see **Figure 3**. These results confirm β-irradiation-induced mechanical deterioration in PCL nanofibers.

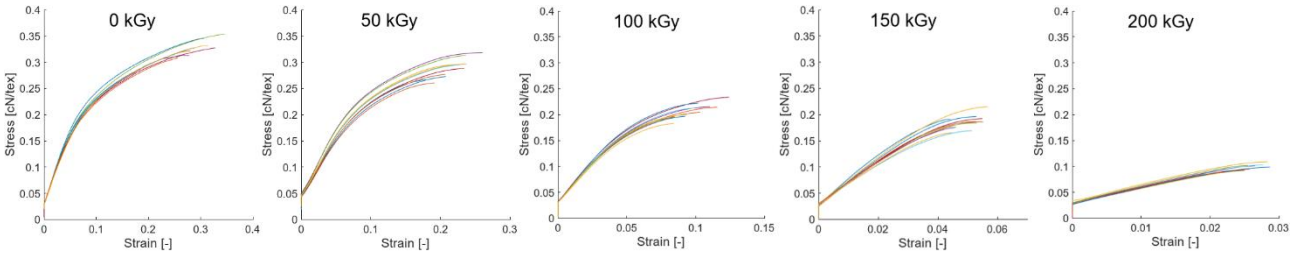


Figure 2 Obtained stress-strain curves for β-irradiated PCL nanofibrous layers.

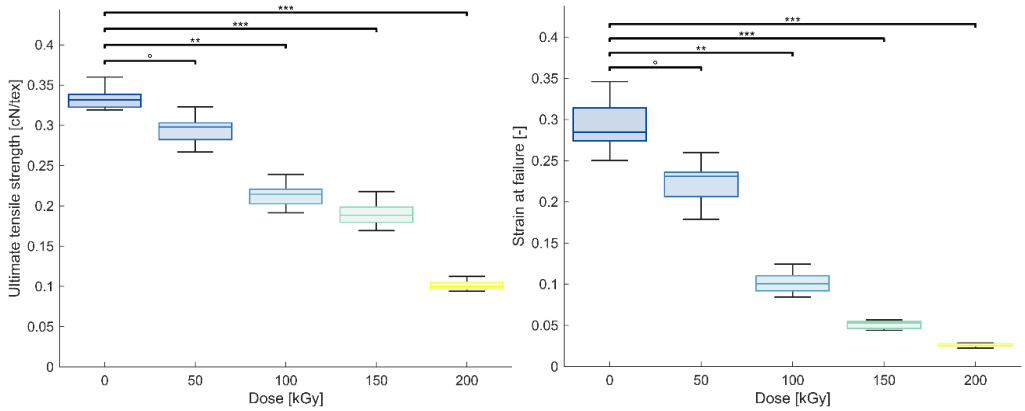


Figure 3 Box plots representing obtained ultimate tensile strength and strain at failure for β-irradiated PCL nanofibres and control sample. The sign ° denotes no statistical significance, *, **, *** denote statistical significance at significance levels of 0.05, 0.01, 0.001 (Kruskal-Wallis).

Morphological and structural analysis

Scanning electron microscopy revealed no visual differences between irradiated and control samples, see **Figure 4**. All samples exhibited a mix of smooth submicron and micron fibers with sporadic bead defects. Despite mechanical degradation, no fiber fractures or surface cracks were observed, unlike UV-weathered macroscopic polymer materials, as described by White [9]. Average fiber diameter analysis showed statistically significant differences, but no meaningful trend can be observed in statistically significant changes of average fibre diameter with respect to obtained dose, see **Figure 5**. Furthermore, effect size expressed by Cohen's d for all samples except 50 kGy proofed to be small ($d < 0.22$). Thermal analysis indicated a gradual increase in crystallinity from 59 % up to 68 % (350 kGy), see

Table 1, with a slight drop at 400 kGy, probably due to inappropriate handling with fragile sample. Secondly melting temperature decreased with obtained dose. These observations suggest chain scissions in amorphous regions allowing for secondary crystallization, as previously described by White [9].

Table 1 Thermal analysis data of β-irradiated PCL nanofibers

Dose [kGy]	*0*	*50*	*100*	*150*	*200*	*250*	*300*	*350*	*400*
Enthalpy of crystallite melting – ΔH_m [J/g]	*79.7*	*81.8*	*83.4*	*88.5*	*90.1*	*89.6*	*90.3*	*92.1*	*90.9*
Degree of crystallinity – X_C [%]	*58.8*	*60.4*	*61.6*	*65.3*	*66.5*	*66.2*	*66.7*	*68*	*67.1*
Melting temperature – T_m [°C]	*62.4*	*60.4*	*61.5*	*62.3*	*62*	*60.3*	*59*	*59*	*58.8*

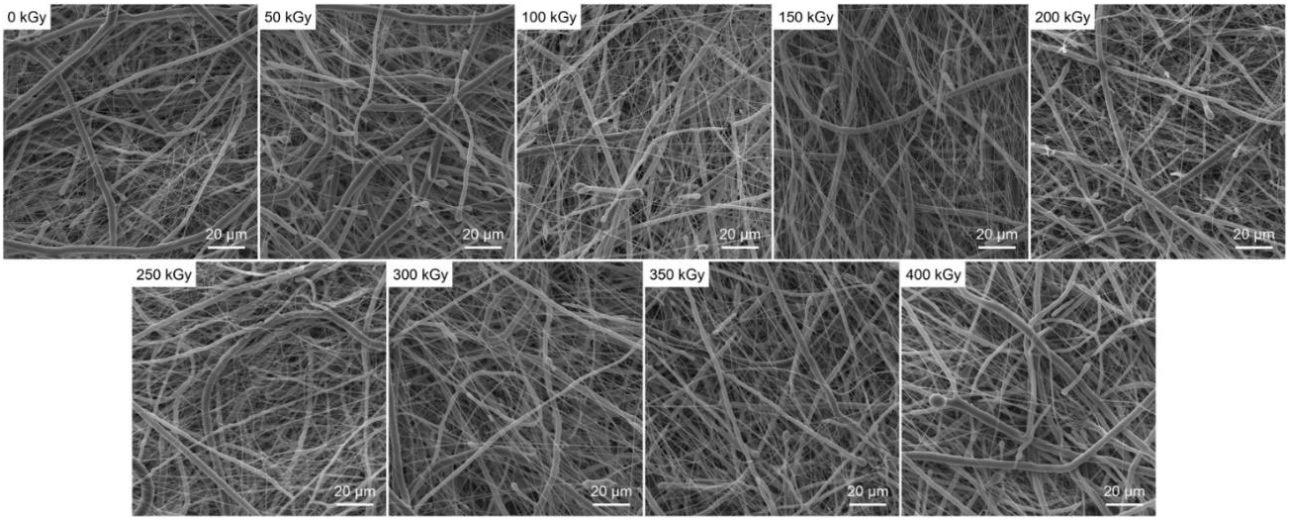
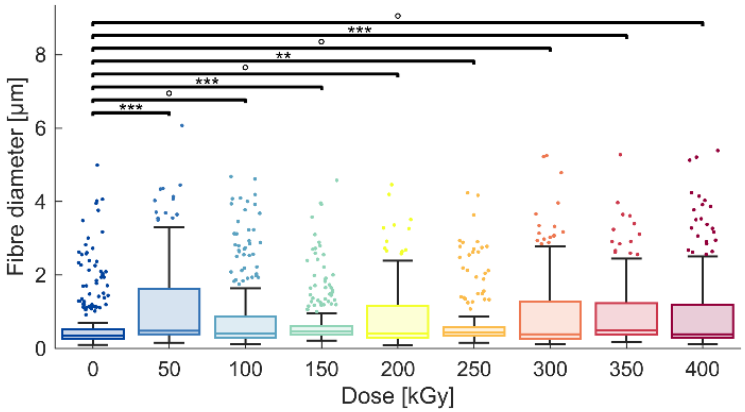


Figure 4 Scanning electron microscope images of PCL fibres irradiated by β-irradiation



Dose	*Average diameter*	*Cohen's d*
0 kGy	0.70 ± 0.83 µm	-
50 kGy	1.06 ± 1.05 µm	0.378
100 kGy	0.87 ± 1.01 µm	0.182
150 kGy	0.71 ± 0.69 µm	0.018
200 kGy	0.79 ± 0.81 µm	0.116
250 kGy	0.66 ± 0.68 µm	0.048
300 kGy	0.88 ± 0.97 µm	0.202
350 kGy	0.88 ± 0.82 µm	0.220
400 kGy	0.90 ± 1.05 µm	0.214

Figure 5 Box plot representing fibre diameters for β-irradiated samples and control sample. The sign ° denotes no statistical significance, *, **, *** denote statistical significance at significance levels of 0.05, 0.01, 0.001 (Kruskal-Wallis). Table representing average fibre diameter and calculated values of Cohen's d to evaluate size effect against control sample.

Cytotoxicity

All of the β-irradiated samples were considered sterile, control sample was sterilized by ethylene oxide. None of the tested samples show any toxic effects after incubation in the DMEM culture medium. All tested materials except 300 kGy sample exhibited more than 90 % cell viability, see **Figure 6**. Nevertheless, cell viability is still above toxicity threshold of 70 %, set by used standard. Notably, the cell viability did not decrease further even for samples with higher doses (350 and 400 kGy). Based on obtained data, β-irradiation at doses up to 400 kGy does not compromise biocompatibility of electrospun PCL nanofibres.

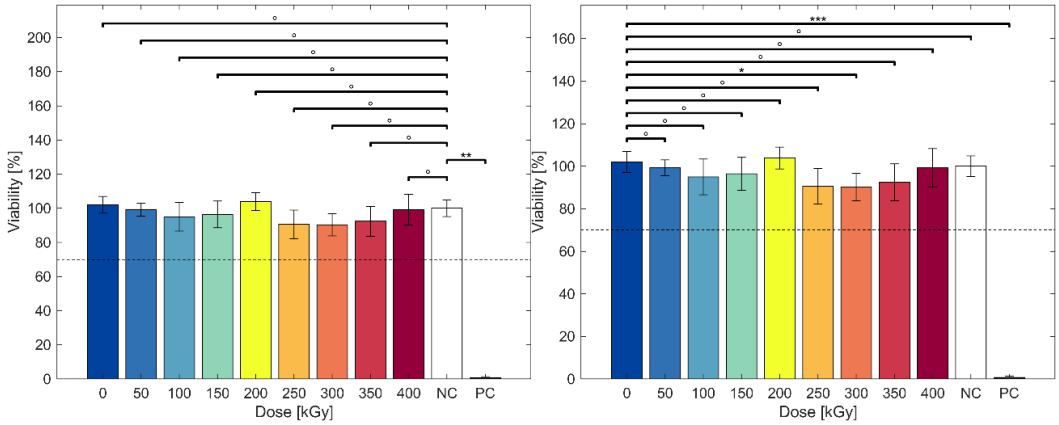


Figure 6 Bar charts representing cell viability of β-irradiated PCL nanofibers. Dashed line denotes viability threshold representing cytotoxicity following EN ISO 10993-5 (855220). The sign ° denotes no statistical significance, *, **, *** denote statistical significance at significance levels of 0.05, 0.01, 0.001 (Kruskal-Wallis).

4. CONCLUSION

This study evaluated the effects of β-irradiation on the morphology, structure, mechanical properties, and biocompatibility of electrospun polycaprolactone nanofibers. PCL was chosen for its biodegradability, biocompatibility, and relevance in biomedical applications. Furthermore, biodegradable material was chosen to lower environmental and health risk connected with potentially harmful products of aging process. Nanofibers were produced via needleless electrospinning, and irradiation was conducted using a microtron MT25 with doses ranging from 0 to 400 kGy in 50 kGy increments. Tensile tests revealed significant reductions in mechanical properties for doses above 50 kGy, confirming β-irradiation as a viable method for accelerated aging studies. SEM showed no visible fiber damage, while thermal analysis highlighted increased crystallinity and reduced melting temperatures due to chain scission in amorphous regions and secondary crystallization. Despite structural and mechanical changes, biocompatibility was unaffected, with all samples showing cell viability above 90 %. This study demonstrates that β-irradiation offers a time-efficient alternative to UV aging, enabling the study of polymer nanofiber aging and its effects on material behaviour in environmental conditions.

ACKNOWLEDGEMENTS

This work was developed thanks to the financial support of the student grant competition project (SGS-2024-4481) from the Technical University of Liberec.

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