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Experimental study on the effect of selected sterilization methods on mechanical properties of polylactide FFF specimens

Abstract

Purpose: Biodegradable polymers are widely used in personalized medical devices or scaffolds for tissue engineering. The manufacturing process should be finished with sterilization procedure. However, it is not clear how the different sterilization methods have an impact on the mechanical strength of the 3D printed parts, like bone models or personalized mechanical devices. This manuscript presents the results of mechanical testing of polylactide based bone models before and after sterilization.

Design/methodology/approach: Polylactide specimens prepared in fused filament fabrication technology were sterilized with different sterilization methods: ultraviolet and ethylene oxide. Mechanical properties were determined by testing tensile strength, Young modulus and toughness.

Findings: The tensile strength of material after sterilization was significantly higher after ethylene oxide sterilization compared to the ultraviolet sterilization, but in both sterilization methods the specimens characterized lower tensile strength and Young modulus when compared to the control. In comparison of toughness results there was no statistically significant differences. The findings are particularly significant in the perspective of using individual implants, bone grafts and dental guides.

Originality: Although FFF 3D printing devices equipped with UV light sterilization options are available, experimental results of the effect of selected sterilization methods on the mechanical strength of additively manufactured parts have not been described. This paper completes the present state of the art on the problem of sterilization of FFF parts from biodegradable materials.

Keywords Mechanical properties, Sterilization, Polylactide, Fused filament fabrication, UV light sterilization, Ethylene oxide sterilization

Paper type Research paper

List of abbreviations

3D – three-dimensional
CO_2 laser – carbon dioxide laser
EtO – ethylene oxide
H_2O_2 – hydrogen peroxide
HDPE – high density polyethylene
kGy – Gray (unit)
MHAp – mackerel fish hydroxyapatite
PA – Peracetic Acid
PCL – polycaprolactone
PLA –polylactic acid
PLCL – poly-L-lactide-co-ε-caprolactone
PLGA – poly(lactide co-glycolide)
TMPTMA – trimethylopropane trimethacrylate
TPU – thermoplastic polyurethane
UV – ultraviolet

1. Introduction

The expansion of the three-dimensional printing technology has resulted in a wide range of applications, e.g. in biomedical applications, for implantology (Singh *et al.*, 2019; Vasamsetty *et al.*, 2020), bone defect replacements (Andrzejewska, 2019; Bose *et al.*, 2013; Ghorbani *et* *al.*, 2020) or personalized mechanical devices (Sapoval *et al.*, 2020; Wesemann *et al.*, 2020). The safety of implantable materials should respect a number of standards related to the biocompatibility, proper mechanical properties but in addition, the microbiological safety aspect seems to be the most important. For this reason, ready-to-use products including personalized medical devices and implantable materials are sterilized.

The mechanical properties of 3D printed bone models or other personalized medical devices are crucial for its application. The above-mentioned properties may be affected by properties on each stage of manufacturing process like storage of materials, environmental factors during processing, postprocessing sterilization. Also different methods of sterilization of polymer materials applied in biomedical solutions are used. The different sterilization methods can affect changes in mechanical properties.

Tipnis and Burgess (2018) recognized and developed methods of sterilization of polymeric materials for biomedical applications. Following methods include: ethylene oxide, radiation, dry and heat steam, H_2O_2 and ozone, also peracetic acid, UV light, microwave, sound waves and pulsed light.

The study conducted by Haim Zada et al. (2019) describes the effect of ethylene oxide sterilization and gamma sterilization on the behavior of Poly-L-lactide-co-ε-caprolactone (PLCL) specimens prepared in the multicycle dip-coating process. The main conclusion of the researchers is the recommendation of EtO sterilization instead of gamma-radiation for PLCL balloon implants. Chen *et al.* (2019) described the stability of specimens made of high density polyethylene (HDPE) and Polyamide 6 and exposed to two sterilization methods - novel vaporized hydrogen peroxide and electron beam processes. The specimens were prepared both by additive manufacturing and by injection molding. The research presented by the authors proved that injection molded specimens were more stable than 3D printed specimens upon sterilization processes.

Ng et al. (2019) reported the effect of electron beam irradiation at room temperature on the properties of compression-formed PLA in combination with fish bone waste mackerel (MHAp) and trimethylpropane trimethacrylate (TMPTMA). The authors noted that when the radiation dose increased, the mechanical properties of the composite improved due to better crosslinking. Meanwhile, in the case of pure PLA, increasing the radiation dose contributed to a decrease in strength.

Artemenko et al. (2012) investigated the influence of sterilization methods such as dry heat, autoclave and UV radiation on chemical and biological properties of plasma polymers. General finding of the work was conclusion that there exists no universal sterilization method that assures preservation of the properties of all kinds of plasma polymers.

Davison et al. (2018) discussed the results of tests performed on specimens of poly(lactide co-glycolide) PLGA prepared by compression molding. Specimens were gamma sterilized at 40 kGy and room temperature or low temperature (-80°C) in a nitrogen atmosphere. The results reported that the molecular weight was significantly reduced, as was the glass transition temperature, which indicates a chain rupture. FTIR reported minor changes in the chemical structure in methyl and carbonyl groups after irradiation. The glass transition temperature changed significantly between irradiation at -80°C and irradiation at 25°C, but this difference was only 1°C. Consequently, the results indicate that the applied sterilization temperature does not affect PLGA when carried out in a nitrogen atmosphere.

Polymeric Tissue Engineering Scaffolds in Yoganarasimha et al. (2019) research were prepared by electrospinning method from polycaprolactone (PCL) was sterilized with Peracetic Acid (PA). The main goal of the study was to determine the effect of the selected sterilization method on the cytotoxicity of PCL scaffolds. It has been shown that the rinsing of scaffolds in 80% ethanol for 30 minutes effectively eliminates toxic PA waste and restores the cytocompatibility.

De Cassan et al. (2019) work presents scaffolds manufactured from PCL were also produced by electrospinning and then sterilized using three methods, i.e. electron beam, gamma radiation and Röntgen radiation. It was shown that the dose of radiation had a significant effect on changes in molecular mass and degree of crystallinity, whereas the type of used radiation had no significant effect on changes in mechanical behaviour.

Preem et al. (2019) reported study of scaffolds manufactured with the PCL electrospinning method. The generated scaffolds were exposed to UV-sterilization, gamma-irradiation, chlorine gas. It was observed that gamma sterilization increased the hardness and elasticity of PCL constructs as a result of increased crystallinity of the polymer.

Rainer et al. (2010) used in research constructions performed with the method of electrospinning from polylactide, which were exposed to soaking in absolute ethanol, dry oven and autoclave treatments, UV irradiation, and hydrogen peroxide gas plasma treatment. The study disclosed that UV irradiation and hydrogen peroxide gas plasma are the most effective sterilization techniques, which ensure sterility of the electrospun scaffolds without affecting the chemical and morphological features.

In view of the work described below, it can be stated that various sterilization methods can be successfully used to sterilize the thermoplastic polymers. Nevertheless, the field of effect of sterilization methods on structural components made by additive manufacturing methods is still not well understood. There are single literature reports demonstrating the proper way of sterilization of porous constructions produced by additive manufacturing methods. Luchini et al. (2021) describes results of sterilization with heat-based methods and sanitizing with various chemical solutions of 3D printed polylactic acid (PLA) or thermoplastic polyurethane (TPU) parts. This study shows that while personal protective equipment is produced using PLA and the traditional infill-based patterns model may be initially sterile, re-sterilization is not possible using methods such as isopropanol, bleach, and/or H_2O_2. In addition, autoclaving is technique typically utilized to sanitize a variety of materials, but it isn't suitable for PLA and TPU 3D printed parts.

In the manuscript Pérez Davila et al. (2021) the analyzed how the most common techniques used to sterilize PLA medical devices are affecting the physicochemical and biocompatible properties of 3D printed items.

It has been observed that EtO sterilization is the most universal and the most widespread method of low-temperature sterilization in large clinical centers (Boiano and Steege, 2015; Sobaszek *et al.*, 1999). Also it is considered to be the method with high effectiveness, low cost. While UV methods are used in small rural clinics (Rutala and Weber, 2015), dental practices (Cumbo *et al.*, 2020) or beauty salons (Sowah and Ahiabor, 2014). Moreover, manufacturers of 3D printers offer devices equipped with the possibility of UV sterilization during printing. Therefore, the objective of this study was to investigate the effect of sterilization methods: UV light and ethylene oxide on the mechanical properties of 3D printed components produced from biodegradable polylactide. The results of testing the mechanical properties of sterilized parts are important for research and development in regenerative medicine and medical devices, which must be biologically safe for users. The preliminary study of mechanical properties that was conducted should result in the most suitable method for sterilization of 3D printed parts, to be used in future studies on the effectivity of sterilization methods.

2. Methodology

Dog-bone shaped specimens were used to determine changes in the mechanical behavior of 3D printed polymeric materials and then sterilized. The geometry and optimal parameters of fused filament fabrication are similar as in further research (Andrzejewska, 2021; Andrzejewska *et al.*, 2019). For this experiment, commercially available 3DXPLA007-EA polylactide (Sigma-Aldrich, Saint Louis, MO, USA) was applied. Specimens were prepared with the method of fused filament fabrication, on a 3D printer Kreator Motion (Krakow, Poland).

Printing of elements was based on the planned density of cross-sectional infill equal to 100% and the angular placement of material fibres in relation to the specimen axis, i.e. +45º/-45º. The specimen shape and geometry based on (ISO 527-2:2012, 2012) standard is presented in Figure 1. However, the 3D printing settings of dog-bone shaped specimen are summarized in Table 1.

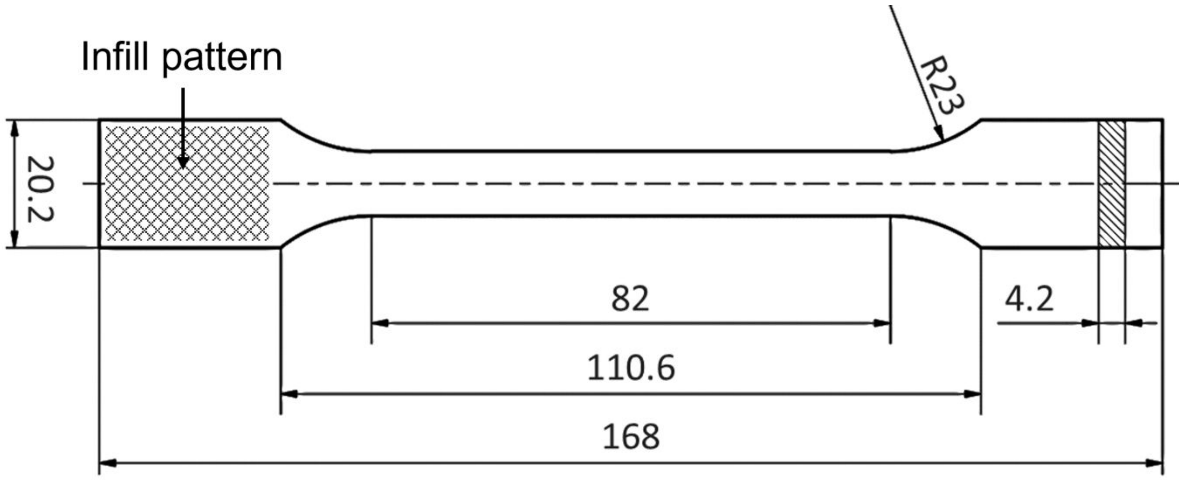

Figure 1. Geometry of dog-bone shaped specimen.

Table 1. 3D printing parameters

No.	Selected parameters	Value
1.	Nozzle temperature	200°C
2.	Bed temperature	65°C
3.	Nozzle diameter	0.4 mm
4.	Filament diameter	1.75 mm
5.	Layer thickness	0.1 mm
6.	Fiber orientation to specimen axis	+45°/-45°
7.	Outline	2
8.	Top/bottom solid layers	6/6

Two methods of sterilization were chosen, i.e. UV light sterilization and ethylene oxide sterilization. The process of sterilization based on “Guideline for Disinfection and Sterilization in Healthcare Facilities” of Centres for Disease Control and Prevention. Sterilization with ultraviolet radiation was conducted in UV-C sterilizer (Activ, Wroclaw, Poland), using UV radiation of 254 nm wavelength. The time of sterilization of the specimens was 30 minutes and the process was established at 60°C. The UV sterilized forms were deposited in a desiccator filled with silica gel for 24 hours. However, the second group of specimens were sterilized with ethylene oxide in Steri-Vac Sterilizer (3M, Saint Paul, MN, USA). The following process parameters were defined: gas concentration - 450 mg/l; temperature - 55°C; relative humidity - 60%; exposure time - 60 min. Subsequently, after exposure to the sterilizing agent, the specimens were subjected to a degasification period lasting 12 hours in the sterilizer chamber. The tests of mechanical properties were performed on the INSTRON ElectroPuls E3000 (Norwood, MA, USA) tensile machine with an electromagnetic actuator of ±3 kN force. The traverse speed of the testing machine was 1 mm/min. Tests of material's mechanical properties to uniaxial tensile strength before and after sterilization with two methods were realized at room temperature. In each group of tests, 5 specimens were subjected to mechanical properties measurements (Andrzejewska *et al.*, 2019; ISO 527-2:2012, 2012)

3. Results and discussion

Three parameters were used in the analysis of the influence of the selected sterilization method on changes in mechanical behavior of the biodegradable material. The tensile mechanical behavior of the sterilized polylactide parts was determined: ultimate tensile strength (σ_m), Young's modulus (E) and toughness i.e. the amount of absorbed energy needed to break the specimen (Q) (Jyoti *et al.*, 2022; Mohammadi *et al.*, 2017; Yang *et al.*, 2019). The toughness is parameter expressed by the Equation (1).

$$Q = \int_t \sigma d\varepsilon \qquad (1)$$

Figure 2 presented representative cases of stress-strain curves of non-sterilized control specimens and specimens exposed to two different methods of sterilization. On the grounds of the presented charts it can be observed that specimens before sterilization were characterized by the greatest value of tensile strength and the greatest elongation. However, the specimens after sterilization, in relation to the selected method, were characterized by reduced strength and elongation in comparison with non-sterilized specimens. Higher values of strength and elongation were reported for specimens after ethylene oxide sterilization. Temperature-induced sterilization contributes to scission of the polymer chain, which results in reduced tensile strength and elongation (Otaguro *et al.*, 2010; Papadimitriou *et al.*, 2021).
In the process of determining the statistical significance of differences, the recorded and calculated values of mechanical parameters were collected and then the results were analyzed. Table 2 summarizes the mean value, standard deviation and median of the determined strength parameters. The coefficient of variation of results received for the three selected parameters was determined for each group of tested specimens. Besides, the statistical significance of differences in results between individual groups of specimens was compared. Estimates of statistical significance of the differences were performed using GraphPad Prism. Comparison of specimens before and after sterilization by two methods was performed by one-way ANOVA test and post-hoc Fisher's LSD test. The analysis was carried out at the significance level of $p < 0.05$ (Norani *et al.*, 2021; Zhu *et al.*, 2021).

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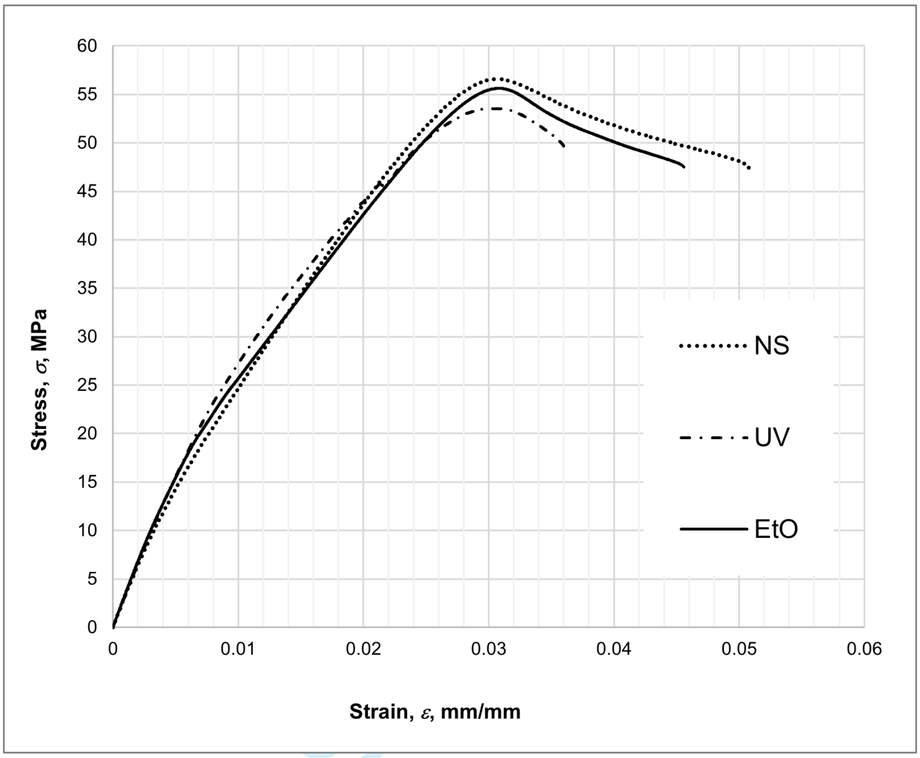

Figure 2. The representative stress–strain curves of the specimens before (NS) and after UV light or EtO sterilization.

Table 2. Calculated values of tensile strength parameters

	Ultimate Tensile Strength, σ_M, MPa		Young modulus, E, MPa		Toughness, Q, MJ/m^3	
PLA part	**Mean ± STD**	**Median**	**Mean ± STD**	**Median**	**Mean ± STD**	**Median**
Non-sterilised	57.92 ± 0.66	58.22	3585.45 ± 123.32	3598.36	1.007 ± 0.004	1.021
UV light-sterilised	53.38 ± 0.16	53.37	3116.28 ± 195.01	3045.35	1.080 ± 0.060	1.049
EtO-sterilised	55.54 ± 0.47	55.55	3121.81 ± 216.14	3121.92	1.059 ± 0.090	1.040

In case of the analysis of the coefficient of variation of results in each group of specimens, the coefficient value was lower than 10% regardless of the analyzed parameter. Statistical comparison of differences in specific parameters between groups of non-sterilized and UV or ethylene oxide sterilized specimens showed statistically significant differences in tensile strength (p-value <0.0001). Furthermore, statistically significant differences in Young modulus were shown in comparison of specimens before and after sterilization by both methods (p-value = 0.0017). However, there were no statistically significant differences in changes in Young modulus between the specimens that were sterilized (p-value = 0.9626). When comparing toughness results, no statistically significant differences were found between samples before and after sterilization with both methods.

Figure 3 shows a comparison of several groups of specimens in relation to parameters reached in a tensile test and calculated on the basis of experimental data.

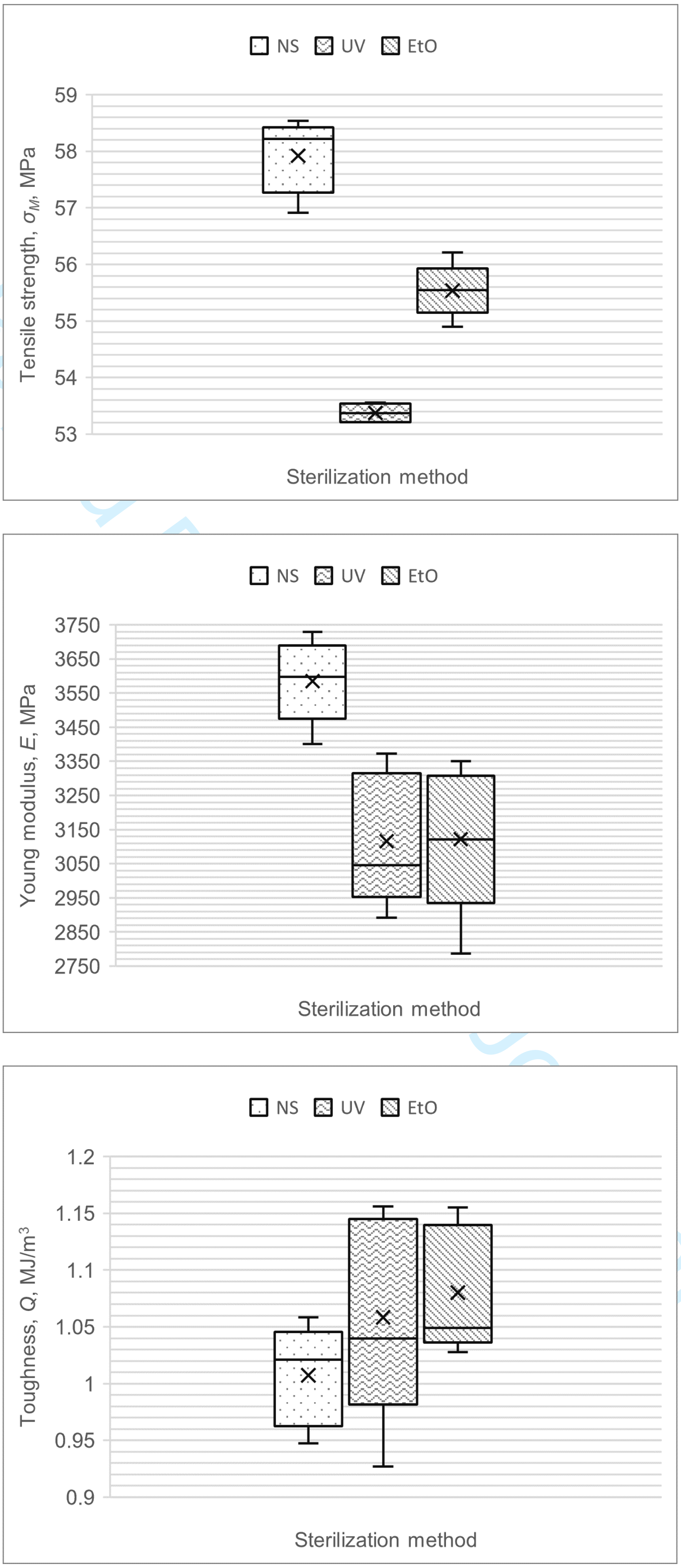

Figure 3. Comparison of mechanical properties: a) tensile strength, b) Young modulus, c) toughness.

Although statistically significant differences in tensile strength values have been shown between non-sterilized and sterilized specimens with different methods, the reduction in strength values does not exceed 2 MPa for EtO sterilization and respectively 4 MPa for UV sterilization. The results obtained after EtO sterilization are very similar to the results obtained by other researchers in publication (Zhao *et al.*, 2019), whereas the expected strength value of the material may be varied and may depend on the method of specimens preparation, grade of material, content of two forms of the monomeric acid (D-or L-lactic acid) etc. The stress and strain values in printed specimens will be affected by the printing temperature and then the sterilization temperature. The effect of heating strongly influences the changes of polymeric bonds in the entire specimen. As noted Grasso *et al.* (2018) stress redistribution is crucial in tests on specimens previously treated with temperatures close to the glass transition temperature of PLA and then cooled.

The important parameter found in tests presented in this research is toughness, which determines the specimen's susceptibility to fracture. In the documents of other scientists, no information was found regarding the determination of toughness parameter, specifically its changes due to the sterilization of biodegradable 3D printed material.

5. Conclusions

The results of the research presented in this paper compared the influence of the method of sterilization on mechanical properties of biodegradable material.

- The obtained results provided, in general, a lower strength of the sterilized specimens.
- The reduction in strength value from 2 to 4 MPa should not be considered as a disincentive to sterilize 3D printed elements.
- The geometry of the specimens was measured before and after sterilization. Changes in geometrical dimensions (cross-sectional area) did not exceed 10%. Value of the cross-sectional area after sterilization was taken for strength calculations. Due to the effect of temperature (close to glass transition temperature), a change in the ordering of polymer chains and crystalline transformations may have occurred, but further studies are necessary to confirm above.

Based on mechanical properties both EtO and UV light sterilization are suitable for sterilizing bone models or personalized medical devices. EtO sterilization results in lower strength loss and is declared in literature as more microbiologically effective than UV. The effectiveness of sterilization 3D printed parts will be evaluated in future research.

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Author Contributions: It is single-authored paper.

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Conflicts of Interest: The authors declare no conflicts of interest.

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Table 1. 3D printing parameters

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8.	Top/bottom solid layers	6/6

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Table. 2. Calculated values of tensile strength parameters

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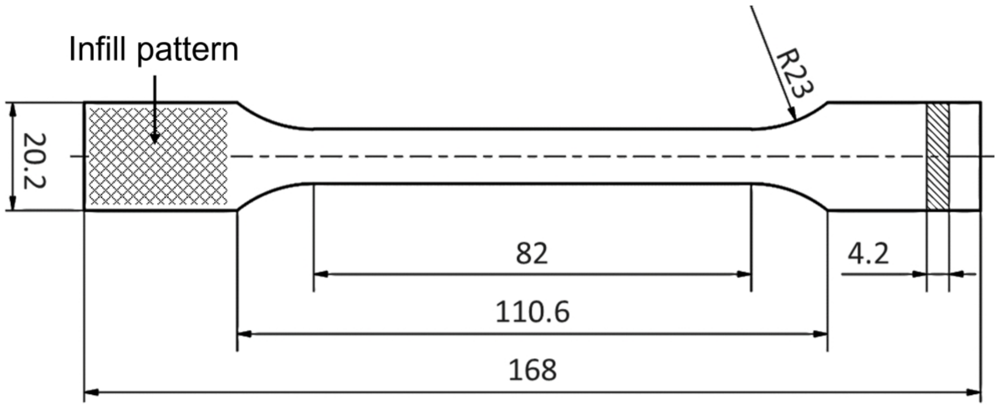
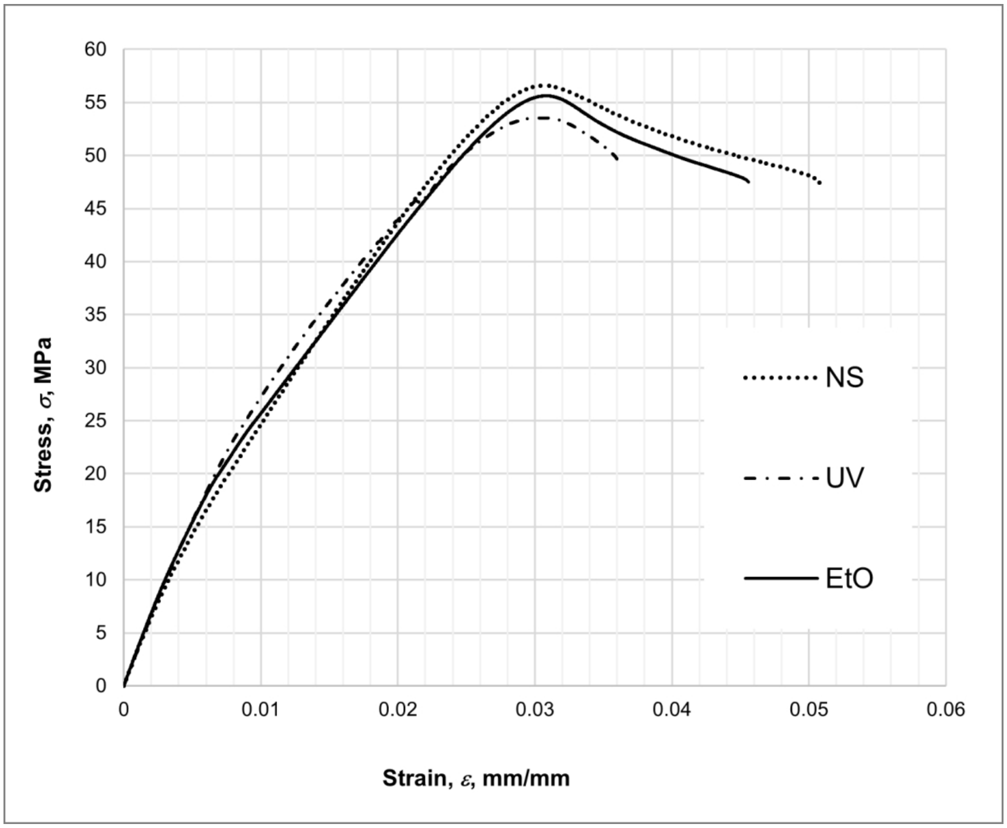


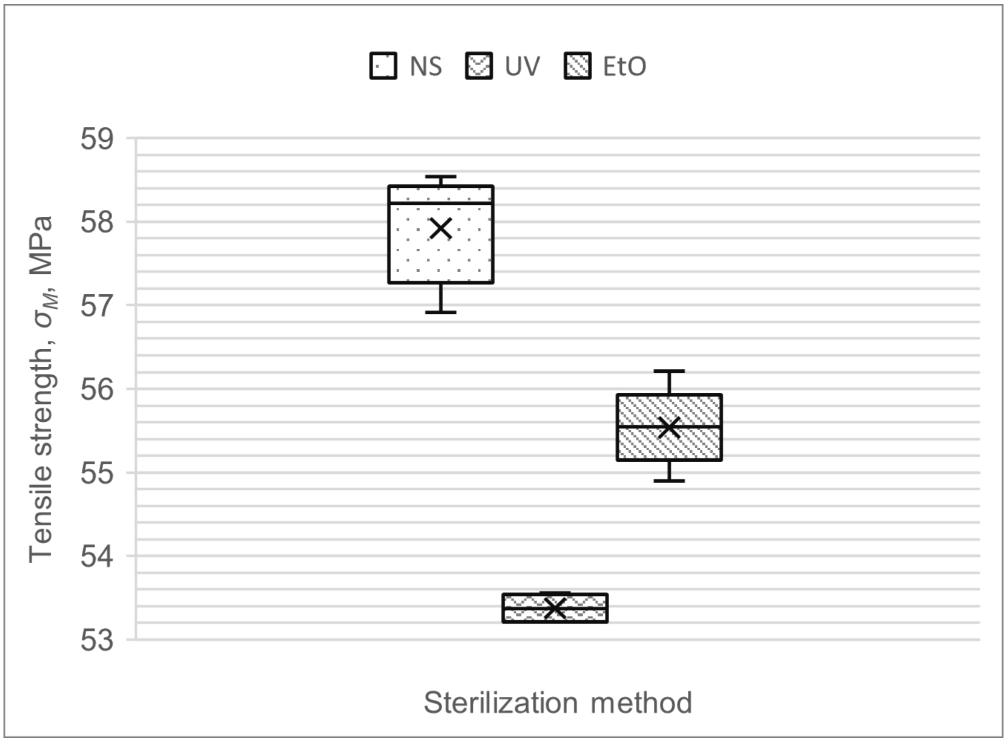
Geometry of dog-bone shaped specimen.

485x196mm (130 x 130 DPI)



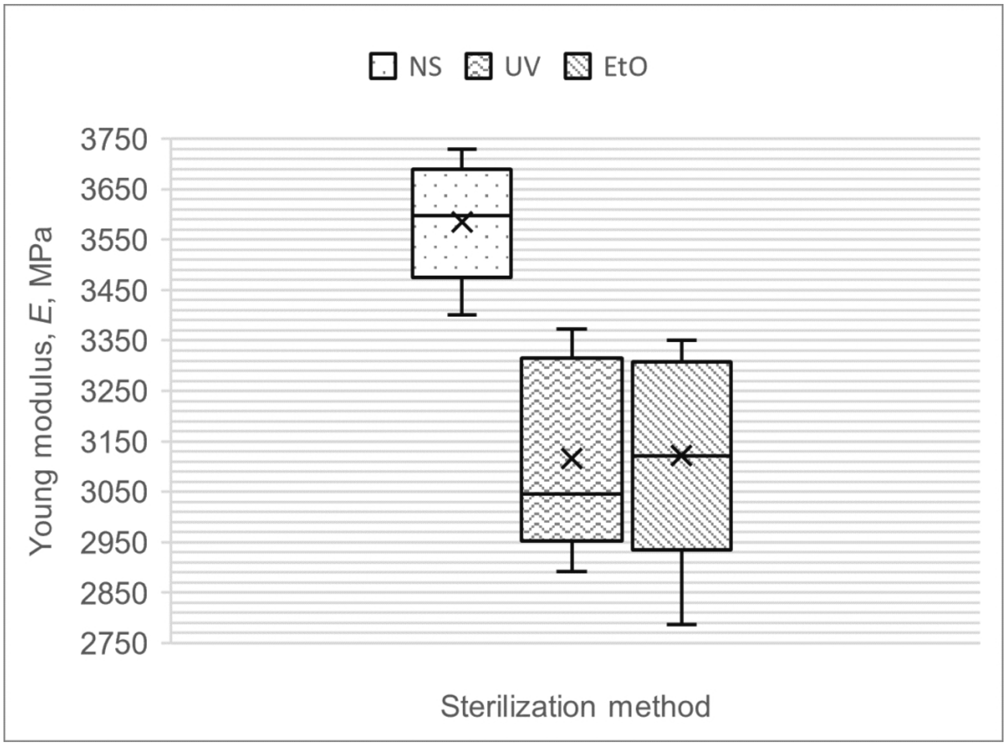
The representative stress–strain curves of the specimens before (NS) and after UV light or EtO sterilization.

314x257mm (130 x 130 DPI)



Comparison of mechanical properties: a) tensile strength, b) Young modulus, c) toughness.

263x193mm (130 x 130 DPI)



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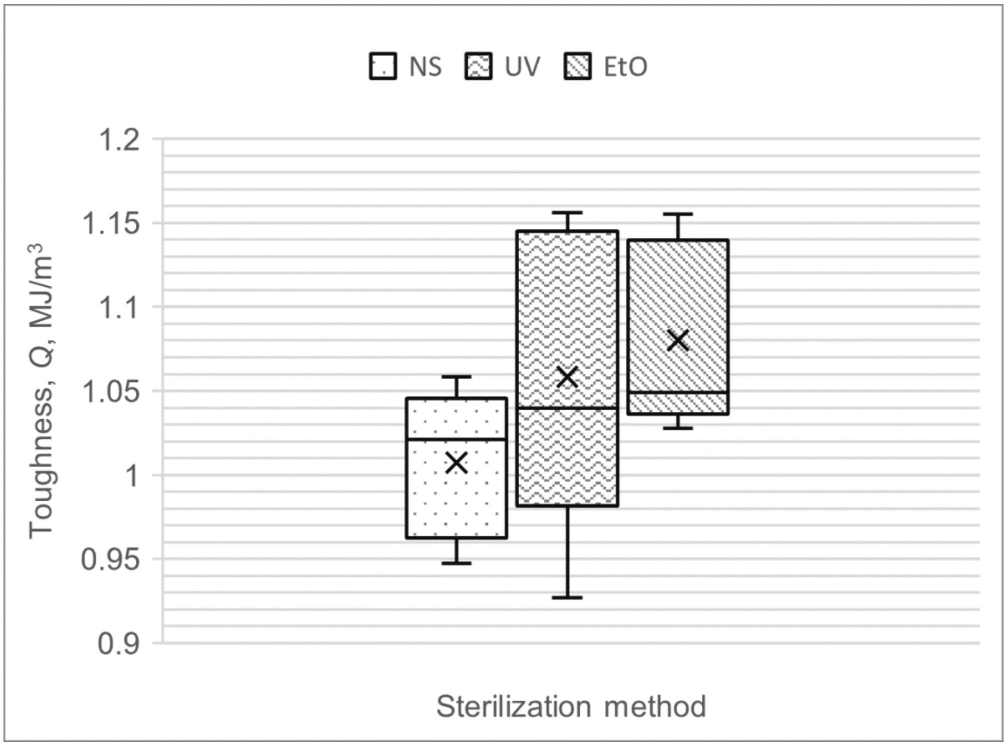

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