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Effects and efficacy of different sterilization and disinfection methods on electrospun drug delivery systems

Liis Preem[a], Ebe Vaarmets[a], Andres Meos[a], Indrek Jõgi[b], Marta Putrinš[c], Tanel Tenson[c], Karin Kogermann[a,*]

[a] Institute of Pharmacy, University of Tartu, Nooruse 1, 50411 Tartu, Estonia
[b] Institute of Physics, University of Tartu, W. Ostwaldi 1, 50411 Tartu, Estonia
[c] Institute of Technology, University of Tartu, Nooruse 1, 50411 Tartu, Estonia

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ABSTRACT

Microbiological quality of a pharmaceutical product is an essential requirement ensuring patient safety, thus effective sterilization/disinfection methods need to be found. The aim of this study was to evaluate the efficacy of different sterilization/disinfection methods on drug-loaded electrospun matrices and the impact of these treatments on the functionality related characteristics of these matrices. The sterilization efficacy of gamma-irradiation, ultraviolet-irradiation, *in situ* generated chlorine gas and low-pressure argon plasma treatment were evaluated on two different chloramphenicol-loaded electrospun matrices using pristine polycaprolactone (PCL) as a carrier polymer or PCL in combination with polyethylene oxide. Drug stability, solid state properties, morphology, mechanical properties, swelling, biodegradation and drug release kinetics were studied before and after the treatments. It was shown that all tested methods help to reduce bioburden and only plasma treated matrices were not sterile. At the same time drug degradation after the treatment can be considerable and depends not only on the susceptibility of the drug to degradation, but also on matrix properties (e.g. the nature of carrier polymers). Even though no morphological changes were observed, gamma sterilization increased the hardness and elasticity of PCL matrices as a result of increased crystallinity of the polymer. Plasma treatment was able to significantly enhance water absorption to otherwise hydrophobic PCL/CAM matrix and had tremendous impact on its drug release kinetics as the drug was instantly released from otherwise prolonged release formulation.

1. Introduction

Electrospinning is a rapidly advancing technology for the production of nano- or microfibers by creation and elongation of electrified fluid jet (Mehta et al., 2019; Reneker and Yarin, 2008; Xue et al., 2017). The applications of electrospun matrices range from biomedicine (wound dressings, tissue engineering scaffolds, drug delivery systems) to physics and material sciences (batteries, capacitors, sensors, filters, protective clothing etc.) (Lu and Ding, 2008). For some of these applications, sterility of a matrix is a critical quality attribute and a key requirement for an adequate and safe performance, most notably for biomedical applications. Contamination of such devices can lead to infection and result in severe complications prolonging or suspending healing with possibly fatal outcomes (Darouiche and Darouiche, 2001).

Microbiological quality can be ensured by either utilizing aseptic manufacturing or different sterilization/disinfection methods. Sterilization is a process that destroys or eliminates all forms of microbial life. In medicine, several sterilization methods are in routine use and described in pharmacopoeias, e.g. heat sterilization (dry heat or steam), ionizing radiation (gamma rays, a beam of electons, or X-rays), gas sterilization (including alkylating and oxidizing agents, e.g ethylene oxide) and membrane filtration. In addition to the accepted sterilization methods, several disinfection methods are in use to eliminate pathogenic microorganism with the exception of bacterial spores, e.g. ultraviolet (UV) irradiation or treatments with ethanol solutions (Rediguieri et al., 2016). Terminal sterilization after product packaging is recommended over other options as this can provide a sterility assurance level that is possible to calculate, validate and control (European Medicines Agency, 2016). Still, the sterilization process cannot be solely relied upon for creating a high-quality product. The manufacturing

* Corresponding author.
E-mail addresses: liis.preem@ut.ee (L. Preem), andres.meos@ut.ee (A. Meos), indrek.jogi@ut.ee (I. Jõgi), marta.putrins@ut.ee (M. Putrinš), tanel.tenson@ut.ee (T. Tenson), karin.kogermann@ut.ee (K. Kogermann).

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conditions must be designed to support minimal contamination during preparation and packaging to increase the sterilization efficacy and reduce endotoxin levels in the end product.

The choice of an appropriate sterilization/disinfection technique depends largely on the object that needs to be decontaminated. Sterilization/disinfection of electrospun drug-loaded matrices brings about many challenges that need to be considered in choosing optimal treatment method. Moreover, the final application also dictates many considerations. For example, tissue scaffolds need to provide specific biomechanical cues required for optimal tissue regeneration (He et al., 2014), thus these matrices cannot lose their mechanical properties after sterilization. Also, the matrix materials are highly important and need to be regarded. Biodegradable polymers with low melting points are often used for electrospinning (Cipitria et al., 2011). Thus, thermal sterilization methods are usually not applicable. Soaking in ethanol solutions has to be disregarded if the matrix polymers are soluble in ethanol or if the matrix is drug-loaded. Gas sterilization is also problematic in case of electrospun matrices as these matrices are known for high surface area and porosity and can adsorb high amount of sterilant gases on their surface or in their structure, thus raising concerns for potential toxicity. For example, ethylene oxide is highly toxic and thus problematic agent for the sterilization of such matrices. Extensive purging steps following sterilization for 48 h at 50 °C, as recommended, could reduce the problem, but might still be inefficient or impractical in case of fiber formulations and lead to plastic deformation if polymers with low melting point are used (Odelius et al., 2008).

On the other hand, there are several methods that are more promising for the sterilization of electrospun fiber matrices. Gamma irradiation is one of the most commonly used terminal sterilization methods after heat sterilization techniques. Not only does it allow sterilization without using elevated temperatures, but by the virtue of its highly penetrative nature, it is possible to sterilize packed products (Silva Aquino, 2012). UV-treatment is often used for the decontamination of air and surfaces due to the low cost and availability of the technology, it is relatively fast and temperatures are low (Cutler and Zimmerman, 2011; Dai et al., 2016b). Sterilization with *in situ* generated chlorine gas is less common, yet practiced in other fields (Lindsey et al., 2017; Phillips et al., 2015; Yang et al., 2013). Plasma sterilization is also an alternative to the traditional methods that does not utilize heat or moisture. Thus, these methods could potentially be used for decontamination of electrospun scaffolds, although their effects still need to be studied to avoid any unanticipated and deleterious effects on matrix properties.

Some studies have already been performed on characterizing the effects of sterilization methods on electrospun polymeric matrices (Rainer et al., 2010; Valente et al., 2016). The effect of ethylene oxide sterilization on electrospun scaffolds constructed from polycaprolactone PCL and a copolymer consisting of polylactide and PCL (PLCL) were investigated for the determination of their mechanical properties, degradation rates and interaction with fibroblasts by Horakova et al. (Horakova et al., 2018). Rediguieri et al. have provided a more thorough review on the impact of sterilization methods on electrospun scaffolds for tissue engineering (Rediguieri et al., 2016). Still, only few of those studies have been conducted on drug-loaded electrospun matrices, concentrating on selected properties and sterilization methods. For example, Thakur et al. studied the effect of ethylene oxide sterilization on antibiotic activity, delivered drug concentration and ability to support human fibroblast growth on poly-L-lactic acid PLLA matrix with mupirocin and lidocaine (Thakur et al., 2008). Thus, more information on the properties of drug-loaded matrices after different sterilization and disinfection processes are needed.

The aim of this study was to compare different sterilization/disinfection methods (gamma irradiation, UV-irradiation, *in situ* generated chlorine gas, low-temperature argon plasma) on various properties (drug stability, solid state, mechanical properties, swelling and biodegradation, drug release) of electrospun drug-loaded matrices. Two different polymeric compositions were studied – polycaprolactone (PCL) alone or in combination with polyethylene oxide (PEO) – with the model antibacterial drug chloramphenicol (CAM). These matrices have been described in a previous publication (Preem et al., 2017). The sterilization/disinfection methods were chosen to accommodate the restrictions coming from the matrix materials and formulation characteristics.

2. Materials and methods

2.1. Materials

Polycaprolactone (PCL) (Mn 80,000), polyethylene oxide (PEO) (Mw ≈ 00,000) and chloramphenicol (CAM) were purchased from Sigma Aldrich. Methanol (gradient grade), chloroform and acetic acid (puriss p.a.) were purchased from Lach-Ner. Commercial chlorine-based bleach (ACE™, Procter&Gamble) for house-hold use was obtained for chlorine gas generation. Dehydrated culture media soya-bean casein digest medium (also known as tryptic soy broth or TSB, BD Bacto™) and fluid thioglycollate medium (Lab M) were reconstituted and sterilized according to the manufacturer's instructions. Phosphate buffered saline (PBS) with pH adjusted to 7.40 was used in drug release, swelling and biodegradation experiments.

2.2. Electrospinning

Preparation of electrospinning solutions and applied conditions have been already published (Preem et al., 2017) and are briefly summarized in Table 1. NanoNC electrospinning robot (model ESR200R Series, Es-robot®) (South Korea) was used for carrying out electrospinning. In all cases, the volume of solution electrospun was 10 mL. Fibers were collected on a ground roller (diameter 9 cm × width 20 cm) covered with an aluminum foil. The spinneret was moving in horizontal direction (distance 14 cm) at the speed of 25 mm/min, whereas the roller speed was 20 rpm, thus allowing to collect uniformly and reproducibly thick fiber matrices. Electrospinning was carried out at ambient conditions (temperature of 22 ± 1 °C and RH of 20 ± 2%).

Electrospun matrices were stored at ambient conditions in plastic ziplock bags until further analysis. To minimize the pre-sterilization bioburden, aluminum foil and the inner surfaces of the electrospinning robot were cleaned with ethanol prior electrospinning, the fibers were stored in clean plastic bags and the bench was kept clean while handling the fibers.

Table 1
Composition of electrospinning solutions and electrospinning parameters.

Formulation	Polymeric composition [w/V% in CF:MET 3:1 V/V]	Drug load [w/w% of fibers]	Solution flow rate [mL/min]	Spinneret and collector distance [cm]	Voltage [kV]
PCL	12.5% PCL	0	1	14	9–10
PCL/CAM	12.5% PCL	4	1	14	9–10
PCL/PEO	10% PCL, 2% PEO	0	2.5	17	14
PCL/PEO/CAM	10% PCL, 2% PEO	4	2.5	17	14

Key: CAM – chloramphenicol; CF – chloroform; MET – methanol; PCL – polycaprolactone; PEO – polyethylene oxide.

2.3. Sterilization

Different sterilization and disinfection methods were used for the treatment of electrospun fiber mats. After sterilization (except gamma sterilization), the fibers were placed with sterile forceps into sterile 50 mL Falcon tubes for storage until further analysis.

2.3.1. UV-sterilization

All electrospun matrices were removed from the foil and cut into appropriate pieces for later testing (1 × 1 cm or 2 × 2 cm) and exposed to UV-irradiation at 254 nm (UV-lamp: Heraeus model TNN 15/35, Germany) at the distance of 15 cm from the lamp for 15 min, 30 min or 1 h on both sides in ambient conditions. UV lamp power was 15 W.

2.3.2. Gamma-irradiation sterilization

All electrospun matrices that were later used for sterility testing were removed from the foil, cut into 1 × 1 cm pieces and transfered to 50 mL Falcon tubes for sterilization. Electrospun matrices that were used in other tests were left on foil and cut into appropriate pieces after sterilization. Gamma-sterilization was carried out by Scandinavian Clinics Estonia OÜ. Irradiation was performed using ^{60}Co radiation source at dose level of ~50 kGy.

2.3.3. Chlorine gas sterilization

Chlorine gas was generated *in situ* by mixing 10 mL of commercial chlorine based bleach with 20 mL of acetic acid in sterilization chamber (250 mL beaker, covered with a rubber glove). The samples to be sterilized were removed from the foil and cut into appropriate pieces (1 × 1 or 2 × 2 cm) and placed into a weighing glass (without the cap) and immediately after mixing the bleach and acid, the weighing glass was put into the sterilization chamber. Chlorine gas is generated from hypochlorites according to the Eq. (1):

$$NaClO + CH_3COOH\ CH_3COONa + H_2O + Cl_2 \quad (1)$$

The generation of chlorine gas was detected visually as an appearance of yellowish gas and by the bleaching of wetted universal indicator paper (Lachema) attached above the liquid level, on the upper part of the sterilization chamber. The samples were kept in a sterilization chamber for 1 or 2 h.

2.3.4. Plasma sterilization

Plasma treatment was carried out with a capacitively coupled plasma reactor Femto-PC (Diener electronic GmbH, Germany) with the generator frequency 13.56 MHz and maximum power of 100 W. The power was kept at 30 W during the treatment which allowed to keep the reactor temperature below 30 °C. The treatment was performed at the pressure of 0.3 mbar with the argon working gas supplied to the reactor with the flow rate of 4 sccm. The floating potential during the plasma treatment was approximately 50 V and no additional bias voltage was applied. The samples were treated from both sides with the treatment time varying from 0.5 min to 2 min. Optical emission spectrum of plasma with and without the electrospun matrices was acquired by UV–VIS spectrometer (OceanOptics 4000 USA).

2.4. Sterility testing

For sterility testing, two different culture media and incubation conditions were used, according to the European pharmacopoeia (9.0): (1) fluid thioglycollate medium primarily suitable for the cultivation of anaerobic bacteria, but also allows the detection of aerobic bacteria; (2) soya-bean casein digest medium (also known as tryptic soy broth, TSB) for the detection of both aerobic bacteria and fungi. Both media were prepared according to the instructions provided by the manufacturer and dispensed into test tubes prior autoclaving. In both cases, the volume of dispensed aliquotes was 5 mL. The diameter of test tubes for fluid thioglycollate medium was 12 mm and for TSB 15 mm. Autoclaving was carried out for 15 min at 121 °C. After the media had cooled to room temperature (RT), both sterilized and unsterilized control fiber samples (1 × 1 cm) were inserted into marked test tubes under aseptic conditions (direct inoculation method). Each sample was tested in triplicate. Positive controls were obtained by inoculating the fluid thioglycollate medium with anaerobic Gram-negative non-spore forming *Fusobacterium nucleatum spp polymorphum*; and inoculating TSB with aerobic Gram-negative non-spore forming *Escherichia coli* MG1655. Untreated media were used as negative controls. Fluid thioglycollate medium was incubated in anaerobic conditions at 30 °C and TSB in aerobic conditions at 20–25 °C for 14 days. If no evidence of microbial growth was found after the incubation period, the sample complied with the test for sterility. If evidence of microbial growth was found, the sample did not comply with the test for sterility. Also, for the test to be valid, no growth could occur in negative control, whereas for positive controls the growth had to occur. All controls produced expected results, thus tests were considered valid. The results were recorded by making photographs of the tubes and analyzed visually.

2.5. Chemical stability of the incorporated drug (CAM)

To determine the drug content in untreated and differently sterilized fiber matrices, high performance liquid chromatography (HPLC) analyzes were performed according to the European Pharmacopoeia method for a related substance CAM sodium succinate. Shimadzu Prominence LC20 with PDA detector (wavelength at 275 nm) equipped with a column Phenomenex Luna C18(2), 250 × 4.6 mm, 5 µm was used. The mobile phase used was 20 g/L solution of phosphoric acid R, methanol R, and water R (5:40:55 V/V/V), flow rate 1.0 mL/min, and injection volume 20 µL. The fiber matrices were cut into 2 × 2 cm pieces, weighed (weighing approximately 0.01 g) and dissolved in chloroform and methanol (3:1 V/V). Comparing drug content in untreated and sterilized fibers provided information about the chemical stability of the drug during different treatments.

2.6. Solid state analysis

2.6.1. Attenuated Total Reflection Fourier Transformed Infrared (ATR-FTIR) Spectroscopy

ATR-FTIR spectroscopy was performed on both untreated and differently sterilized electrospun matrices using an IRPrestige-21 spectrophotometer (Shimadzu Corp., Kyoto, Japan) and Specac Golden Gate Single Reflection ATR crystal (Specac Ltd., Orpington, UK). The spectra were collected between 600 and 4000 cm^{-1} and each spectrum was the average of 60 scans. All spectra were normalised and baseline corrected.

2.6.2. Differential Scanning Calorimetry (DSC)

PerkinElmer DSC4000 was used for DSC measurements. Differently treated and untreated electrospun fibers with a sample mass of approximately 2 mg were analyzed under 20 mL/min dry nitrogen purge at a heating/cooling rate of 10 °C/min from 0 to 180 °C and 180 to 0 °C. The fibers were inserted into crimped aluminum pans without pinholes during analysis. Indium was used for DSC calibration. Obtained DSC curves were normalized to a sample mass.

2.7. Morphology

Electrospun fibers were observed under scanning electron microscopy (SEM) (Zeiss EVO 15 MA, Germany) with 10000× magnification to assess fiber morphology and diameter. For that, randomly selected areas of the fiber mats were mounted on aluminum stubs and magnetron-sputter coated with a 3-nm platinum layer prior to microscopy.

Table 2
Sterility or non-sterility of tested electrospun fiber matrices after different treatments. For each treatment a number of samples exhibiting microbial growth out of three replicates is presented. Note: no growth was observed in negative controls; growth was observed in positive controls.

Sample	Untreated	UV 15 min	UV 30 min	UV 60 min	Gamma radiation	Cl_2 1 h	Cl_2 2 h	Plasma 0.5 min	Plasma 1 min	Plasma 2 min
PCL in TSB	3/3	0/3	0/3	0/3	0/3	0/3	0/3	1/3	0/3	1/3
PCL in FTG	3/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3
PCL/PEO in TSB	3/3	0/3	0/3	0/3	0/3	0/3	0/3	1/3	1/3	1/3
PCL/PEO in FTG	3/3	1/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3

Key: FTG – fluid thioglycollate medium; PCL – polycaprolactone; PEO – polyethylene oxide; TSB – tryptic soy broth.

2.8. Texture analysis

The mechanical properties of untreated and differently sterilized electrospun matrices were measured with a puncture test using CT3 Texture Analyzer (Brookfield, USA) equipped with a 10 kg load cell. 2 × 2 cm pieces of fibers were used for analysis and their thicknesses were measured with Precision-Micrometer 533.501 (Scala messzeuge, Germany) with the resolution of 0.1 µm at 4 different points. The samples were secured between a film support fixture (TA-FSF) and punctured with a stainless steel cylinder probe (TA-42, diameter 3 mm): trigger load 5 g, and test speed of 1.00 mm/s. The target distance 18 mm was chosen so all samples were punctured during the measurement. Measurements were performed at ambient conditions (22 ± 2 °C and 19% RH). The applied force (N) and distance of the probe (mm) were recorded as the probe deformed the sample and hardness (N), deformation at hardness (mm) and hardness work done (mJ) were calculated.

2.9. Swelling and biodegradation

2 × 2 cm samples (n = 3) were cut from the untreated and differently sterilized fiber matrices and weighed, then immersed into 20 mL of PBS at 37 °C for 24 h. After that, the samples were tapped dry with a filter paper to remove free surface water and weighed to determine the swelling index (Nazemi et al., 2014). The samples were then placed back to PBS and incubated for another six days at 37 °C. The samples were then rinsed in deionized water, air-dried and re-weighed for calculating the weight loss and hence to estimate the biodegradation.

2.10. Drug release

For determining the *in vitro* drug release of CAM from untreated and differently sterilized electrospun fiber matrices, 2 × 2 cm samples (n = 3) were cut from the matrices. These were weighed, placed into 20 mL of PBS (pH 7.40) at 37 °C in 50 mL Falcon tubes. The tubes were placed into dissolution apparatus vessel (Dissolution system 2100, Distek Inc., NJ, USA) containing deionized water and maintained at 37 °C with a thermostat. The tubes were rotated by the paddles at the speed of approximately 75 rpm. Aliquots of 2 mL were removed and replaced with the same amount of PBS at set time points. Drug release was monitored up to one week for PCL/CAM fiber mats and 2 h for PCL/PEO/CAM fiber mats. The aliquots were analyzed using UV-spectroscopy (Shimadzu UV-1800) at the wavelength of maximum absorption (λ = 278 nm) of CAM.

2.11. Statistical analysis

Results are expressed as an arithmetic mean ± standard deviation (SD). Statistical analysis was performed by applying two sample *t*-tests assuming equal or unequal variances depending on the results of the prior F-test with MS Excel 2013 software ($p < 0.05$). In case of multiple comparisons, Holm's method was used for adjusting p-values. The mean diameters of the fibers (n = 150) were calculated using ImageJ software. Drug release profiles of untreated and differently treated matrices were compared by calculating difference (f_1) and similarity (f_2) factors. Time-points included to the analysis were from 0 to the last sampling point where cumulative amount of drug released did not exceed 85%. The profiles were concluded different if $f_1 > 15$ and $f_2 < 50$ (Center for Drug Evaluation Research, 1997).

3. Results

3.1. Sterilization efficacy

Sterility testing was carried out only with blank fiber matrices to understand purely sterilization effects and not to confound it with inherent antimicrobial properties of drug-loaded matrices. Results from sterility testing confirmed that all untreated fiber samples were contaminated and resulted in microbial growth in both growth conditions (Table 2).

The untreated fibers were not handled in aseptic conditions prior testing, but still care was taken to minimize the bioburden as explained (see paragraph 2.2). Despite the precautions, these were not enough to ensure sterility. Both gamma irradiation and chlorine gas treatments for 1 and 2 h were able to effectively sterilize the samples (Table 2). UV-radiation was also an effective method for decontaminating electrospun matrices, although the shortest treatment time of 15 min on both sides gave unreliable results as one sample in FTG medium still exhibited microbial growth (Fig. 1). Longer exposure times were able to sterilize the samples (30 min per side and 1 h, respectively).

Low-pressure argon plasma treatment was able to reduce the bioburden as seen from the reduced number of contaminated samples compared with untreated samples (Table 2), but was still ineffective sterilization method independent on the treatment times used in this

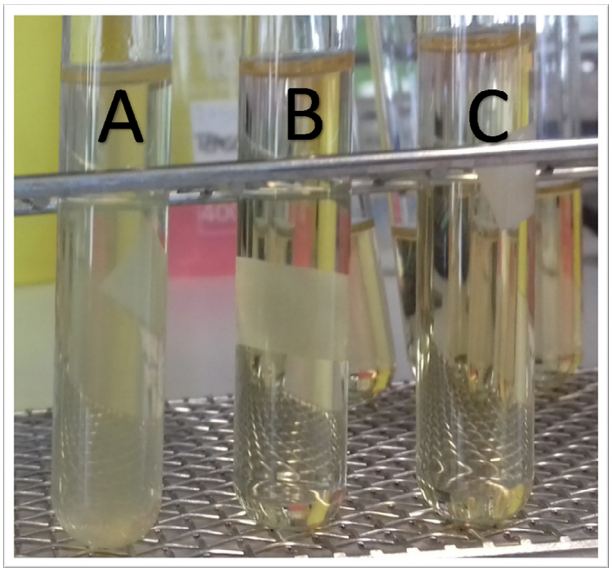

Fig. 1. Sterility test results (fluid thioglycollate medium) of three replicates of PCL/PEO fiber matrices treated with ultraviolet (UV) radiation for 15 min both sides: (A) turbidity due to microbial growth, (B-C) clear medium, no microbial growth. Key: PCL – polycaprolactone; PEO – polyethylene oxide.

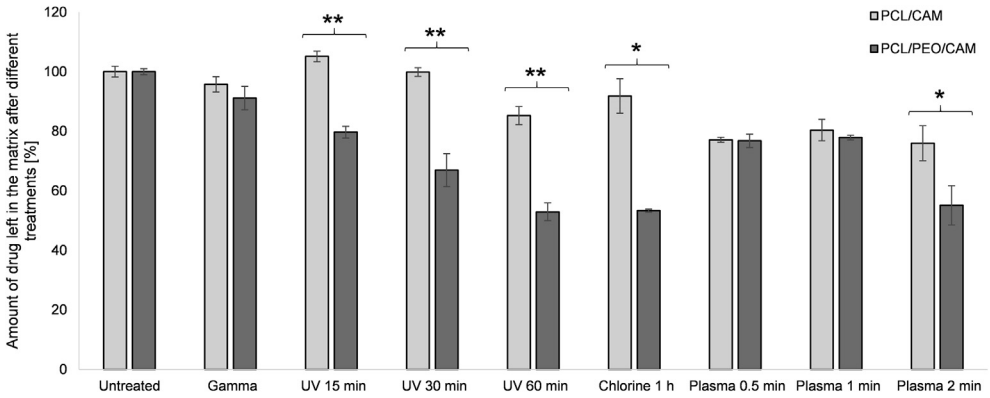

Fig. 2. Chemical stability of CAM in untreated and differently treated electrospun fiber matrices: gamma irradiation, UV-treatment for 15, 30 and 60 min on both sides, *in situ* generated chlorine gas treatment for 1 h and argon plasma treatment for 0.5 min, 1 min and 2 min on both sides. Asterisks depict statistical significance of differences between the drug content in treated PCL/CAM and PCL/PEO/CAM fiber matrices: $^{*}p < 0.05$; $^{**}p < 0.001$. Key: CAM – chloramphenicol; PCL – polycaprolactone; PEO – polyethylene oxide.

study (30 s, 60 s and 120 s). In further analyzes, the shortest effective sterilization time was used for UV and chlorine gas treatments (30 min per side and 1 h, respectively) and 1 min treatment with argon plasma.

3.2. Chemical stability of the drug

Sterilization of drug-loaded products can result in the loss of active ingredient due to degradation. To understand the extent of this possible degradation and determine the stability of drug-loaded fiber matrices after sterilization, the drug content of untreated and differently treated matrices was analyzed with HPLC. The results revealed that sterilization process can indeed lower the amount of drug present in the matrices, but more interestingly, the extent of this loss is largely influenced by the polymeric composition of the matrix (Fig. 2).

In case of UV-sterilized, chlorine gas sterilized and 2 min plasma-sterilized PCL/CAM and PCL/PEO/CAM matrices, there were statistically significant differences in the remaining drug content. With all these methods, the CAM loss was significantly greater in PCL/PEO/CAM matrices. Statistically significant loss of drug was detected in case of PCL/CAM matrices treated with UV for 60 min on both sides and with all plasma treatments. As for PCL/PEO/CAM matrices, all sterilization methods resulted in statistically significant loss of drug. Gamma sterilization had the least detrimental effects on PCL/PEO/CAM matrices drug content and from that point of view would be the most favorable sterilization method.

3.3. Solid state changes

Electrospinning has been recognized as a technique for the preparation of amorphous form of a drug. Due to the rapid evaporation of solvents, the drug has too little time to crystallize (Verreck et al., 2003). This has been shown to be true also for CAM in our PCL and PCL/PEO matrices (Preem et al., 2017). It appears that different sterilization/disinfection treatments do not affect the solid state form of CAM (Fig. 3). Slight changes appear in the peak position of ν_{as}(C-Cl) of CAM (Si et al., 2009) in the spectra of PCL/CAM matrices treated with plasma and chlorine gas (shift from 810 to 816 cm^{-1}) and UV-treatment for 30 min (shift to 814 cm^{-1}), which could indicate recrystallization. On the other hand, the position of ν(C=O) peak (Si et al., 2009) in spectrum remained at 1686 cm^{-1} after all treatments, suggesting that at least partially CAM was still present in an amorphous form in the matrices. Also, peaks related to the ring deformation (649 cm^{-1}) and stretch (1563 cm^{-1}) (Si et al., 2009) were not visible in spectra, further indicating amorphousness. No new peaks or loss of existing peaks were observed in the spectra of matrices after sterilization, neither related to the drug nor the polymers. Thus, it is unlikely that any chemical bonds were formed or broken during these procedures.

It is possible that crystallinity of semicrystalline polymers changes during certain sterilization procedures. Both PCL and PEO have a semicrystalline nature. As crystallinity is a key parameter affecting drug release, mechanical properties, biodegradation rate and thus the overall performance of a scaffold or a drug delivery system, it is necessary to determine if crystallinity indeed changes. Alterations in the intensities of some peaks corresponding to the crystalline/amorphous polymer regions can provide some insight to this matter. For example, increased ratio of crystalline and amorphous C=O stretch (1724 vs 1731 cm^{-1}) or decreased intensity of peak at 2943 cm^{-1} related to amorphous form can indicate increased crystallinity (Yang et al., 2014). Neither changes were observed in sterilized matrices, thus not indicating altered crystallinity of PCL (Fig. 3). DSC was performed to study this matter more in-depth and see whether it supports our FTIR results.

DSC results can be seen in Table 3 and Supplementary Figs. S1 and S2. Gamma sterilization increased PCL melting temperature in all four matrices compared to the untreated matrices, whereas in PCL/PEO and PCL/PEO/CAM matrices, this was accompanied with the loss of PEO peak/shoulder. Although polymer melting temperatures were not greatly affected by other treatments, changes were observed in crystallization temperatures. Gamma sterilization increased crystallization temperatures of both CAM-loaded fiber matrices, although these temperatures were relatively similar or even lower with blank matrices. Crystallization temperatures of argon plasma treated matrices deviated the most compared to the untreated matrices. No CAM melting endotherm nor recrystallization peaks were apparent on the thermograms, most likely due to CAM dissolution in melted polymers, as shown before (Preem et al., 2017).

3.4. Effect on the morphology

Morphology and structural properties of electrospun matrices are important for many, if not all, applications. Also, changes in morphology could bring about other changes, e.g. in mechanical properties (Wong et al., 2008), cell attachment and proliferation (Badami et al., 2006; Lowery et al., 2010), but also in the drug release (Xie and Buschle-Diller, 2010). Thus, it is crucial to study the effects of sterilization methods on matrix morphology. SEM micrographs were taken before and after sterilization and fiber morphologies were compared visually for the presence of possible changes (fusion or breaking of fibers etc) and also fiber diameter analysis was performed. The results revealed that visually, treatments did not cause any visible damage to the fiber structure (Fig. 4).

Diameter analysis revealed that none of the treatments brought about any statistically significant changes in the mean fiber diameters (Table 4). Thus, no gross changes in fiber morphologies were observed, although it must be noted that relatively large deviations in fiber diameters could also make it difficult to detect subtle changes.

Although the treatments did not visually change the structures of electrospun fiber matrices, it was of interest to understand whether the mechanical properties and other relevant material/matrix-related properties were altered.

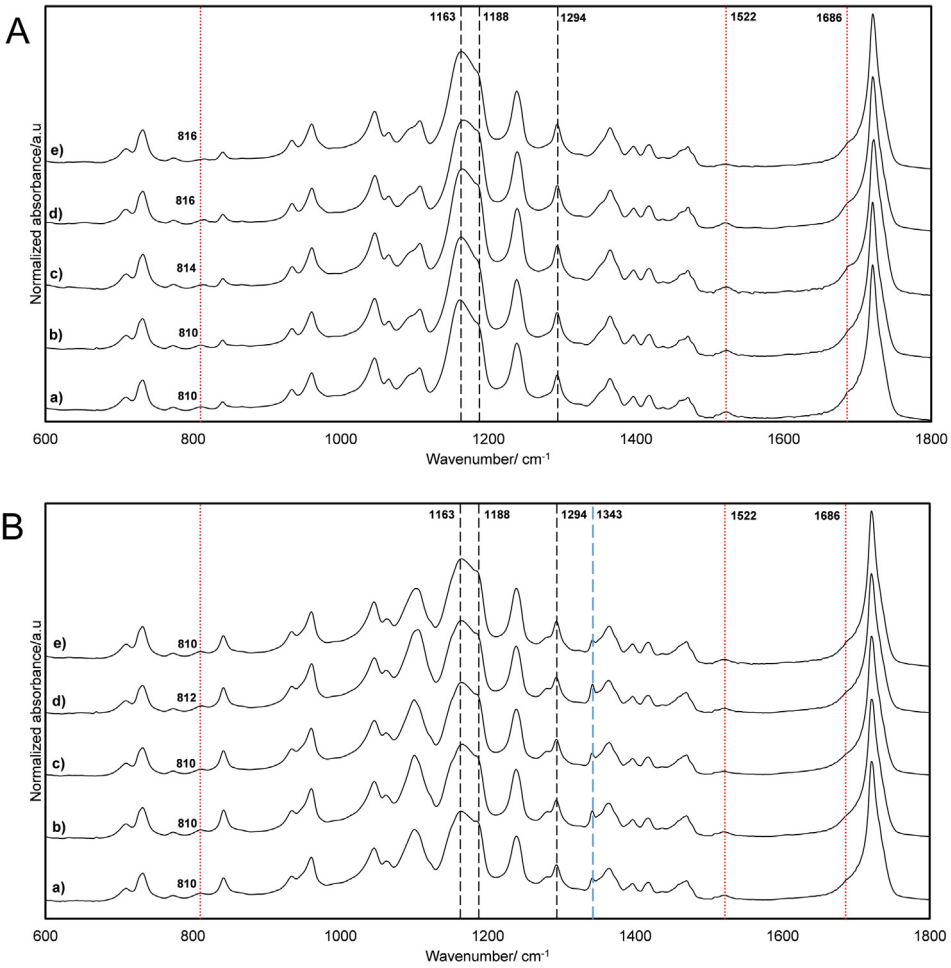

Fig. 3. ATR-FTIR spectra of untreated and differently treated PCL/CAM (A) and PCL/PEO/CAM (B) electrospun fiber matrices: a) untreated; b) gamma irradiation; c) UV treatment for 30 min on both sides; d) *in situ* generated chlorine gas treatment for 1 h; e) argon plasma treatment for 1 min on both sides. Red dotted lines represent positions of CAM bands, black dashed lines represent positions of PCL bands and blue dashed line represents a position of PEO band. Key: CAM – chloramphenicol; PCL – polycaprolactone; PEO – polyethylene oxide.

Table 3
Thermal properties of untreated and differently treated (gamma irradiation, UV-treatment for 30 min on both sides, *in situ* generated chlorine gas treatment for 1 h and argon plasma treatment for 1 min on both sides) electrospun fiber matrices measured using differential scanning calorimetry (DSC).

Fiber matrix	Treatment	Onset temperature of melting [°C]	Peak temperature of melting [°C]	Enthalpy of fusion [J/g]	Peak temperature of crystallization [°C]
PCL	Untreated	57.4 ± 0.7	60.4 ± 0.1	72.5 ± 14.3	26.0 ± 0.4
	Gamma	59.1 ± 0.3	61.6 ± 0.2	88.7 ± 1.9	25.2 ± 0.8
	UV 30 min	58.3	60.4	84.3	28.2
	Chlorine gas	57.3	60.1	67.5	24.7
	Plasma 1 min	57.5	60.7	66.7	35.3
PCL/CAM	Untreated	57.1 ± 0.4	60.5 ± 0.1	77.7 ± 10.4	21.3 ± 0.1
	Gamma	58.4 ± 0.4	61.1 ± 0.1	84.3 ± 7.3	25.9 ± 0.3
	UV 30 min	58.3	60.4	84.3	24.0
	Chlorine gas	58.3	60.5	68.5	22.1
	Plasma 1 min	57.2	60.6	72.4	30.8
PCL/PEO	Untreated	56.1 ± 0.1	59.8 ± 0.1	86.1 ± 3.1	31.2 ± 0.2
	Gamma	58.3 ± 0.4	61.4 ± 0.1	106.4 ± 2.8	28.3 ± 0.5
	UV 30 min	58.1	60.5	84.2	30.3
	Chlorine gas	56.6	60.2	83.8	22.1
	Plasma 1 min	56.2	59.9	99.1	34.5
PCL/PEO/CAM	Untreated	55.5 ± 0.1	59.6 ± 0.1	84.0 ± 9.5	24.0 ± 0.2
	Gamma	57.7 ± 0.1	60.8 ± 0.1	102.3 ± 7.8	29.7 ± 0.2
	UV 30 min	55.7	59.5	88.3	25.8
	Chlorine gas	54.2	59.8	77.8	23.7
	Plasma 1 min	55.0	59.5	97.9	29.7

Key: CAM – chloramphenicol; PCL – polycaprolactone; PEO – polyethylene oxide.

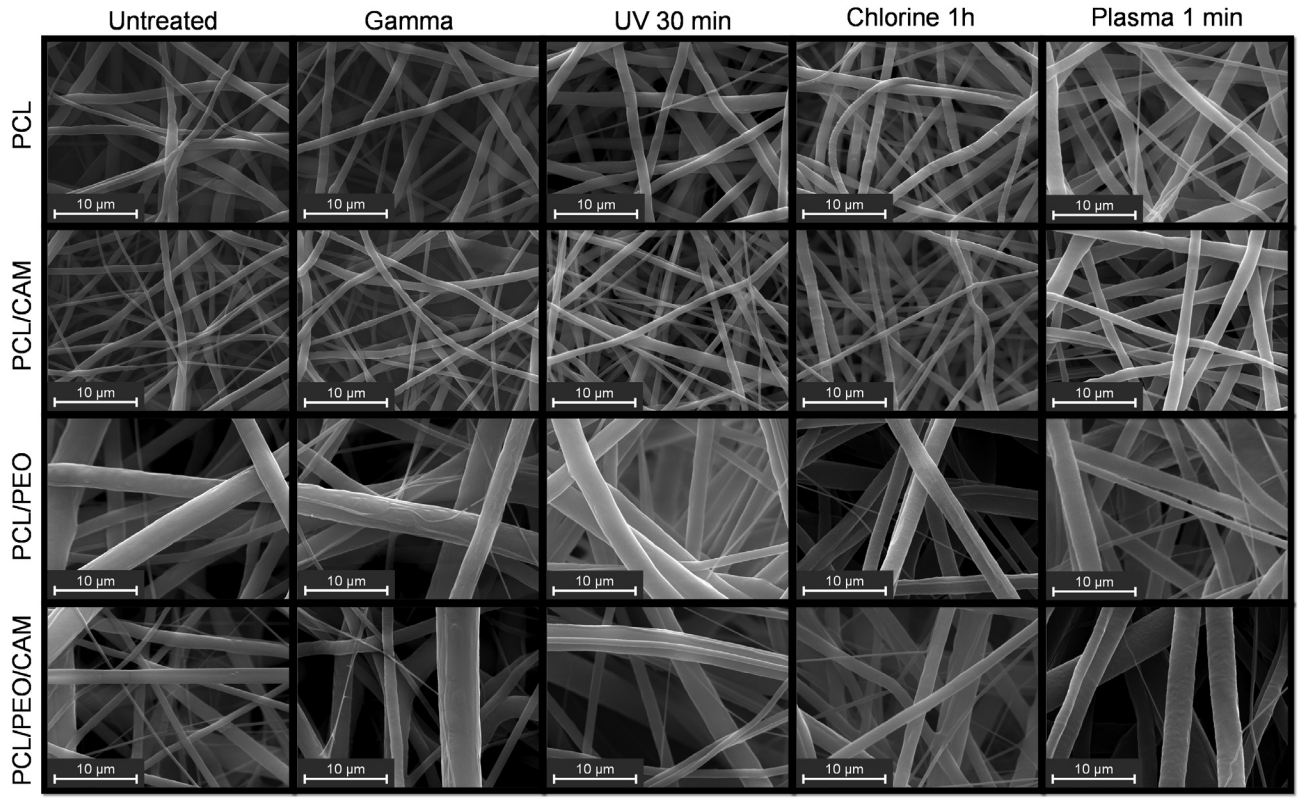

Fig. 4. Scanning electron microscopy (SEM) images (x10000 magnification) of untreated and differently treated (gamma irradiation, UV-treatment for 30 min on both sides, *in situ* generated chlorine gas treatment for 1 h and argon plasma treatment for 1 min on both sides) PCL, PCL/CAM, PCL/PEO and PCL/PEO/CAM electrospun fiber matrices. Key: CAM – chloramphenicol; PCL – polycaprolactone; PEO – polyethylene oxide.

3.5. Effect on mechanical properties

The mechanical properties of PCL and PCL/CAM matrices benefitted from gamma sterilization (Fig. 5, Supplementary Table S1). The hardness increased together with the deformation at hardness, although the latter was statistically significant only for PCL/CAM matrices. Thus, the matrices became both stronger and more elastic. Interestingly, plasma treatment caused statistically significant increase in deformation at hardness of PCL/CAM matrix, but not its hardness. Other treatments did not significantly affect the mechanical properties of these matrices (Fig. 5).

Gamma sterilization also increased the hardness work done of PCL/PEO/CAM matrix, although other changes brought about by gamma sterilization were not statistically significant. For PCL/PEO and PCL/PEO/CAM matrices, UV-treatment had more pronounced effects on the mechanical properties. Interestingly, the hardness, deformation at hardness and hardness work done all decreased after 30 min treatment of PCL/PEO matrices (only the latter being statistically significant change), but increased if the same matrices were drug-loaded (deformation at hardness not statistically significant). As seen, irradiation treatments had greater effect on mechanical properties of electrospun matrices and depending both on the polymers and the drug, the resulting effects can be different. Hence, sterilization processes can both enhance and impair mechanical properties of electrospun matrices.

3.6. Effect on swelling and loss of mass

Remarkable differences were seen in the swelling behavior between PCL (PCL/CAM) and PCL/PEO (PCL/PEO/CAM) matrices (Fig. 6A) and slight differences also occurred between CAM-loaded and blank PCL matrices, as reported before (Preem et al., 2017). PCL/PEO blank and drug-loaded matrices exhibited similar capacities to quickly absorb buffer and this does not change with different sterilization/disinfection treatments. Still, there seems to be a tendency that plasma treatment somewhat decreases the swelling of these matrices, but this is not statistically significant. On the contrary, there is statistically significant increase in the swelling index with both blank PCL and PCL/CAM matrices treated with argon plasma. The resulting swelling indices of plasma treated PCL matrices were even higher compared to any of the PCL/PEO matrices. Other treatments did not result in statistically significant changes in swelling, although gamma treatment seemed to increase the swelling of PCL.

After one week, minimal loss of mass was seen with PCL matrices (0.3–1.9), independent of the treatment. Similar, although slightly

Table 4
Mean diameters (±standard deviation) (µm) of untreated and differently treated (gamma irradiation, UV-treatment for 30 min on both sides, *in situ* generated chlorine gas treatment for 1 h and argon plasma treatment for 1 min on both sides) electrospun fibers.

Fiber matrix	Untreated	Gamma	UV30	Chlorine 1 h	Plasma 1 min
PCL	1.00 ± 0.39	0.95 ± 0.36	1.09 ± 0.58	0.91 ± 0.36	1.08 ± 0.53
PCL/CAM	0.73 ± 0.31	0.74 ± 0.29	0.75 ± 0.32	0.80 ± 0.27	0.82 ± 0.47
PCL/PEO	1.86 ± 0.94	1.93 ± 1.23	1.67 ± 1.04	1.75 ± 1.22	1.82 ± 0.96
PCL/PEO/CAM	1.61 ± 1.12	1.67 ± 1.29	1.61 ± 1.07	1.72 ± 1.18	1.51 ± 1.16

Key: CAM – chloramphenicol; PCL – polycaprolactone; PEO – polyethylene oxide.

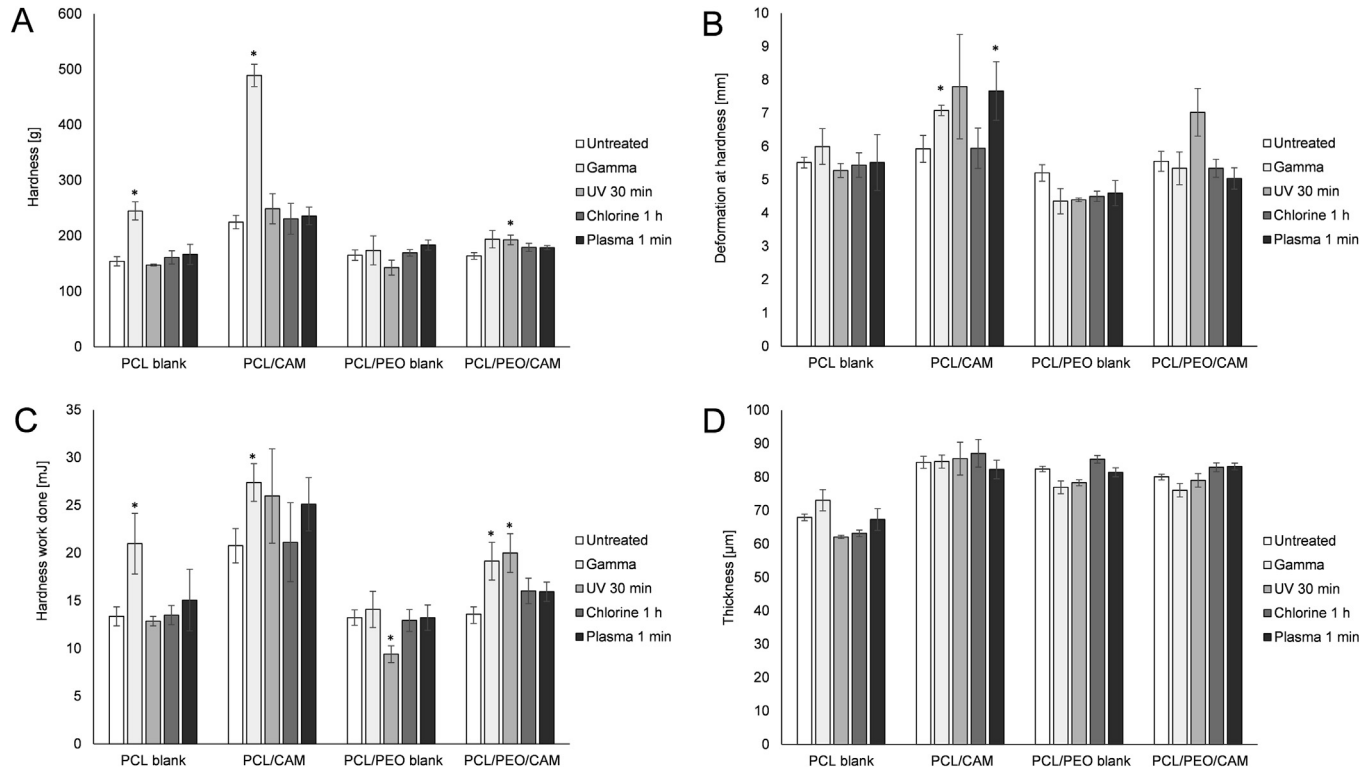

Fig. 5. Mechanical properties and thickness of untreated and differently treated (gamma irradiation, UV-treatment for 30 min on both sides, *in situ* generated chlorine gas treatment for 1 h and argon plasma treatment for 1 min on both sides) PCL, PCL/CAM, PCL/PEO and PCL/PEO/CAM electrospun fiber matrices: hardness (A); deformation at hardness (B); hardness work done (C) and thickness (D). Asterisks depict statistical significance of differences in mechanical properties between untreated and differently treated fiber matrices ($p < 0.05$). Key: CAM – chloramphenicol; PCL – polycaprolactone; PEO – polyethylene oxide.

higher was the loss of mass of drug-loaded PCL/CAM matrices (3.6–5.5%) (Fig. 6B). This is expected, as with these matrices, drug release occurs in parallel with possible biodegradation and loss of mass depends on both. Similar trend was seen with PCL/PEO and PCL/PEO/CAM matrices, where the drug-loaded matrices exhibited higher loss of mass. Compared to untreated matrices, small, although statistically significant increase in the loss of mass was seen with both blank and drug-loaded PCL/PEO matrices treated with UV light for 30 min per side (11.2 ± 0.2% vs 12.7 ± 0.5% and 15.5 ± 0.4% vs 16.7 ± 0.1%, respectively), and also those treated with chlorine gas (11.2 ± 0.2% vs 15.0 ± 0.3% and 15.5 ± 0.4% vs 17.2 ± 0.3%, respectively).

3.7. Effect on drug release

No statistically significant differences were seen in the drug release behavior between untreated and chlorine treated PCL/CAM matrices (difference factor $f_1 = 9.6$; similarity factor $f_2 = 66.2$). On the other hand, after gamma treatment, the release was faster ($f_1 = 16.0$; $f_2 = 59.8$) and after UV-treatment, slightly slower ($f_1 = 15.4$; $f_2 = 58.2$). Still, the most dramatic change was seen after argon plasma treatment where prolonged release was lost and practically all of the drug was released instantly (Fig. 7A).

In case of PCL/PEO/CAM matrices, the shape of the release curves does not change after different treatments and most of the drug is rapidly released within the first 5–10 min. Only chlorine treated matrices deviated noticeably as more drug within the same time-period was

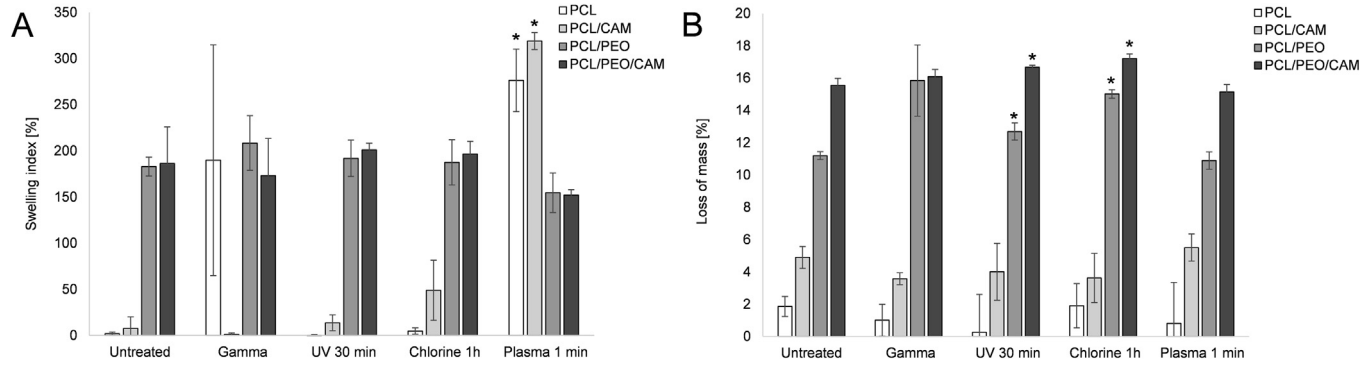

Fig. 6. Swelling indices (A) and loss of mass (B) of untreated and differently treated (gamma irradiation, UV-treatment for 30 min on both sides, *in situ* generated chlorine gas treatment for 1 h and argon plasma treatment for 1 min on both sides) PCL, PCL/CAM, PCL/PEO and PCL/PEO/CAM electrospun fiber matrices. Asterisks depict statistical significance of differences between untreated and differently treated fiber matrices ($p < 0.05$). Key: CAM – chloramphenicol; PCL – polycaprolactone; PEO – polyethylene oxide.

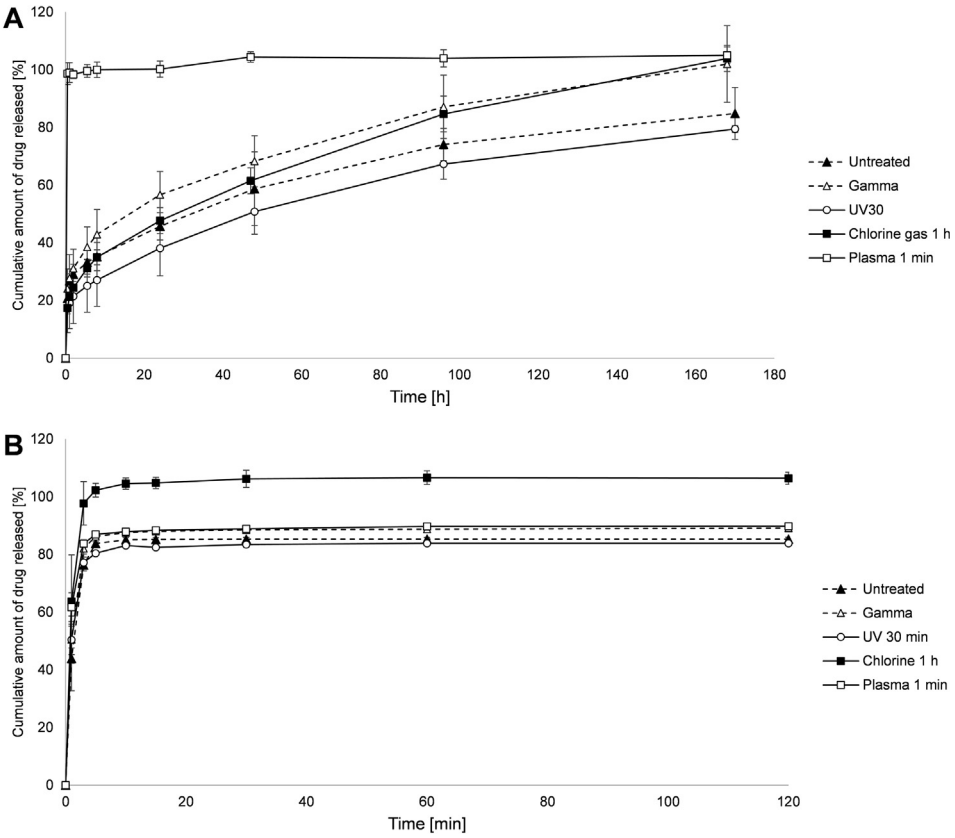

Fig. 7. Drug release profiles of untreated and differently treated (gamma irradiation, UV-treatment for 30 min on both sides, *in situ* generated chlorine gas treatment for 1 h and argon plasma treatment for 1 min on both sides) PCL/CAM fiber matrices (A) and PCL/PEO/CAM fiber matrices (B). HPLC results of drug degradation were taken into account for calculating theoretical 100% that could be released. Key: CAM – chloramphenicol; PCL – polycaprolactone, PEO – polyethylene oxide.

released compared to untreated matrices (Fig. 7B).

4. Discussion

4.1. Sterilization efficacy

There are different sterilization and disinfection methods to choose from to decontaminate electrospun fiber matrices, but their efficacy needs to be proven. Gamma irradiation at the dose of 50 kGy sufficiently sterilized PCL and PCL/PEO electrospun matrices in our study. There have been studies to evaluate the effects of gamma sterilization on electrospun PCL (Augustine et al., 2015; Bosworth et al., 2012; Cottam et al., 2009) or polylactic acid (PLA) (Valente et al., 2016) scaffolds, and also vancomycin HCl loaded PCL microspheres (Sarıgöl et al., 2017), using doses ranging from 15 to 65 kGy. However, in only one of these publications the sterilization efficacy was reported, concluding that doses below 35 kGy may not completely eliminate microorganisms from PCL scaffolds (Augustine et al., 2015). Prevailing minimum dose used for sterilization is 25 kGy, but higher doses may thus be necessary, although it is also known that sterilization efficacy depends on the pre-sterilization bioburden, which is often not discussed.

Chlorine gas treatment proved also to be effective, although, as mentioned gas sterilization has its disadvantages when nanofibrous matrices are concerned due to a high surface area and possibility of residual gas inclusion in the fiber mats. Horakova et al. saw that although no chemical changes were observed with FT-IR after ethylene oxide treatment of PCL and poly-L-lactide-co-ε-caprolactone (PLCL) electrospun vascular grafts, cell proliferation on these mats was slower compared to the grafts treated with ethanol, which could result from toxic residues (Horakova et al., 2018). Whether similar problems could arise from chlorine treated mats, still needs to be studied.

Similar to our study, Dai et al. were also able to sterilize electrospun PCL matrix with 30 min UV-treatment per side (Dai et al., 2016a). PLLA matrix has been effectively sterilized with 20 min UV-treatment (Rainer et al., 2010). At the same time, three dimensional (3D)-printed PCL stents needed at least 2 h treatment to ensure sterility (Guerra et al., 2018), thus the geometry of the object also needs to be considered.

The low-pressure plasma device working with argon can treat the surfaces by the energetic argon ions, excited states of argon atoms and optical emission ranging from infrared to vacuum ultraviolet (VUV) (Denis et al., 2012; Moisan et al., 2002, 2001; Yang et al., 2009). The precise conditions determine which mechanisms are important for sterilization. It has been suggested that the sterilizing effect during the first minutes of plasma treatment is mainly by UV and VUV emission (Moisan et al., 2002, 2001). In addition to UV/VUV emission, the energetic argon ions, high-energy excited states of argon atoms and the trace amount of reactive gases (O_2, H_2O etc.) may also increase the sterilizing effect (Denis et al., 2012; Kylián et al., 2009; Yang et al., 2009). Low-temperature argon plasma was able to sterilize polyethylene terephthalate (PET) sheets contaminated with *Pseudomonas aeruginosa* in less than 1 min while more than 2 min was required when using afterglow of argon plasma where only argon excited states could reach (Yang et al., 2009). Holy et al. managed to sterilize poly(lactic-co-glycolic acid) (PLGA) scaffolds using an argon plasma for 4 min and at 100 W (Holy et al., 2000). On the contrary, it has been reported that argon plasma treatments are not able to completely sterilize the PCL films and increasing treatment time is inefficient (Ghobeira et al., 2017). Thus, there are conflicting results about the efficacy of argon plasma sterilization in the literature and although it proved to be ineffective in our study, plasma technology should not be disregarded as using other process conditions/plasmas could help to effectively sterilize electrospun matrices.

4.2. Drug content

Drug-loaded matrices need to preserve the integrity of the active ingredient after sterilization, thus reducing the amount of possibly toxic

degradation products and ensuring therapeutic efficacy. Remarkable differences occurred with two different polymeric matrices containing the same drug. One supposed reason for these differences is the hydrophobic nature of PCL matrices and hydrophilic nature of PCL/PEO matrices as explained in more depth in Preem et al. (Preem et al., 2017), which can result in different moisture content. As the presence of water is known to hasten drug degradation through several proposed mechanisms (Szakonyi and Zelkó, 2012), this could explain why drug loss was greater in hydrophilic matrices. Similarly, formulation composition and resulting microenvironment has been shown to impact CAM degradation before, as for solid powder and ointment formulations the degradation and its extent during gamma sterilization differ (Hong and Altorfer, n.d.).

Other structural differences of PCL/CAM and PCL/PEO/CAM matrices could also be responsible for or contribute to the different susceptibilities of the drug to degradation. Differences were seen in fiber morphologies as PCL/CAM fibers had below micron mean diameter and hence could be considered as nanofibers, whereas PCL/PEO/CAM fibers were microfibers. The mean pore size was also different, as shown before (Preem et al., 2017). Due to larger pores between PCL/PEO/CAM fibers, gases, like chlorine in this study, could penetrate the matrix more easily and thus have a better access to the drug deeper inside the matrix and cause more degradation.

Gamma treatment caused the least degradation of the drug and it has been shown before to be suitable for the sterilization of CAM loaded pharmaceutical preparations due to low level of CAM radiolysis at irradiation dose of 25 kGy, moreover, the radiolysis products that are generated are safe for human health (Hong et al., 2002). Statistically significant loss of drug detected in case of PCL/CAM matrices treated with UV for 60 min on both sides and with all plasma treatments can be explained by photolytic degradation of CAM (Bakare-Odunola et al., 2009) as VUV is generated in the process of plasma treatment.

4.3. Solid state changes

In addition to degradation, solid state of both the drug and used excipients can be altered after sterilization. Still, FT-IR results did not reveal any great changes in the spectra of differently treated matrices. Augustine et al. saw the appearance of two additional peaks in the IR spectrum of gamma irradiated PCL matrices – one broad peak at 3402 to 3440 cm^{-1} and another one at 1586 to 1593 cm^{-1}, indicating cleavage of ester bonds (Augustine et al., 2015) – neither of those peaks appeared in our gamma treated matrices. Similar to our results Bosworth et al. did not observe any alterations in the functional groups or chemical composition of gamma irradiated PCL matrices using doses up to 45 kGy (Bosworth et al., 2012). It has also been shown that UV treatment for 3 h did not cause any chemical alteration in PCL films (Ghobeira et al., 2017).

Polymer crystallinity plays a significant role in shaping many important properties of electrospun matrices. For example, when PCL matrices were gamma irradiated to the doses up to 35 kGy, the crystallinity of the polymer increased and more ordered arrangement of crystallites were formed, whereas at 65 kGy, it slightly decreased (Augustine et al., 2015). They proposed that with lower doses, chain scissioning resulted in smaller fragments that could spatially rearrange themselves towards new crystalline zones, whereas at higher doses cross-linking took place changing regularly arranged crystallites into non-arranged ones. Increase in the melting temperature and enthalpy of fusion of gamma irradiated PCL matrices, indicating increased crystallinity, have also been reported (Bosworth et al., 2012). Crystallinity of UV-treated PCL stents has been shown to increase as treatment time increased from 2 to 16 h (Guerra et al., 2018). DSC analyses were performed in addition to FTIR experiments to get more insight into the crystallinity. In our study, 30 min UV-treatment may have been short enough to avoid such changes in crystallinity. The crystallization temperature can increase due to nucleating effect of impurities or additives (Mucha et al., 2015), and is affected by polymer molecular weight (Ou-Yang et al., 1997). Although only minor degradation of CAM was observed with gamma irradiation, the crystallization could have been enhanced by the degradation products. Crystallization temperatures of plasma treated matrices deviated the most compared to the untreated matrices. As this was seen equally with blank and drug-loaded matrices, drug degradation products alone cannot explain the phenomenon and most likely changes in polymer structure have also occurred.

4.4. Morphology and mechanical properties

Morphology of the electrospun fibers can sometimes change in the course of sterilization (Valente et al., 2016). PLA fibers lost their preferential orientation after ethylene oxide sterilization, but not after exposure to gamma or UV-radiation (Valente et al., 2016). Also, it has been reported that gamma irradiation does not cause any damage to the electrospun PCL matrices in the dose range of 15–65 kGy (Augustine et al., 2015; Bosworth et al., 2012). Some breaking of PCL fibers has been shown after UV treatment for 5 days (Bajsić et al., 2016). No morphological changes were apparent in PCL films treated with UV for 3 h (Ghobeira et al., 2017). Without the use of additional bias voltage argon plasma has been shown not to cause any changes in PCL film surface roughness due to lack of etching effect, which is however present if extended air plasma treatment is used (Ghobeira et al., 2017).

Two competitive processes occur during gamma sterilization – mechanical strength reducing chain scissioning and crosslinking which increases the mechanical strength. From texture analysis, it appears that crosslinking effects are predominant. Cottam et al. found that gamma irradiation increased both yield stress and failure stress of PCL films and also explained it with crosslinking effects (Cottam et al., 2009). As crystalline polymers have better tensile properties than amorphous ones, changes in crystallinity should also bring about changes in mechanical properties. This nicely correlated with our solid state analyses results which revealed that increased crystallinity of matrices was observed after gamma sterilization. Augustine et al. reported that gamma irradiation up to 35 kGy increased crystallinity and also tensile strength of electrospun PCL fibers, whereas higher dose (65 kGy) resulted in lower tensile strength due to reduced crystallinity (Augustine et al., 2015). Interestingly, Bosworth et al. reported loss in mechanical strength of PCL fibers after gamma irradiation and explained it with lowering of the molecular weight due to chain scissioning which contributed more to the mechanical properties than increase in crystallinity (Bosworth et al., 2012).

UV-treatment for 30 min impaired the mechanical properties of blank PCL/PEO matrices, whereas enhanced those of drug-loaded PCL/PEO matrices. This could indicate that the drug or its degradation products aided the UV-induced photocrosslinking, whereas if no drug is present, polymer(s) may have gone through some changes impairing their mechanical properties, i.e chain scissioning. It is known that PEO can undergo chemical and physical changes due to UV-irradiation and photoreactions are induced by structural defects, impurities or additives (Kaczmarek et al., 2001; Ochoa Machiste et al., 2005).

4.5. Behavior in biorelevant media

It is also important to consider how interactions with aqueous environment mimicking physiological buffers could change in the course of sterilization/disinfection. This does not only affect how much exudate a wound dressing is able to absorb or how long it takes for the matrix to degrade *in vivo* or in the environment after use. But it can also affect the drug release profile, one of the most important parameters for a drug delivery system.

PCL is a hydrophobic polymer and its hydrophobicity is a key property affecting the drug release. As shown by the addition of PEO, a hydrophilic polymer, changes in wettability can change a slow release matrix into a rapid release matrix (Preem et al., 2017). In fact, this

Table 5
Effects caused by different sterilization/disinfection methods on various properties of PCL/CAM and PCL/PEO/CAM matrices.

	Gamma irradiation (~50 kGy)		UV-irradiation (30 min)		Chlorine gas (1 h)		Plasma (1 min)	
	PCL/CAM	PCL/PEO/CAM	PCL/CAM	PCL/PEO/CAM	PCL/CAM	PCL/PEO/CAM	PCL/CAM	PCL/PEO/CAM
Sterility*	Sterile	Sterile	Sterile	Sterile	Sterile	Sterile	Sterile	Not sterile
Drug stability	NE	Unstable	NE	Unstable	NE	Unstable	Unstable	Unstable
Drug solid state	NE	NE	NE	NE	NE	NE	NE	NE
Polymer crystallinity	↑	↑	NE	NE	NE	NE	NE	NE
Morphology	NE	NE	NE	NE	NE	NE	NE	NE
Hardness	↑	NE	NE	↑	NE	NE	↑	NE
Swelling	NE	NE	NE	NE	NE	NE	↑	NE
Loss of mass	NE	NE	NE	↑	NE	↑	NE	NE
Drug release	(↑)	NE	(↓)	NE	NE	↑	↑	NE

Keys: CAM – chloramphenicol; PCL – polycaprolactone; PEO – polyethylene oxide; NE – no effect; *sterility tested using non-drug-loaded fiber matrices; arrow denotes the effect; parentheses indicate small effect size.

effect was also seen after plasma sterilization – the drug release rate was dramatically increased. Plasma is often used to modify the surface properties, most notably to decrease the water contact angle and improve the wettability of the samples (Morent et al., 2011). Unlike untreated PCL and PCL/CAM matrices, plasma treated matrices were instantly wetted when placed in contact with a buffer solution and swelling indices were remarkably increased. Decreased water contact angle of PCL after plasma treatment could be due to the increased number of carboxyl group end chains on the treated surfaces (Ghobeira et al., 2017). As PCL/PEO and PCL/PEO/CAM matrices were already hydrophilic, plasma treatment did not have such effects on those matrices.

High variability was seen in the swelling of gamma treated PCL matrices, where some samples exhibited high buffer absorption, indicating reduced contact angle. Similarly to plasma treatment, this was also reflected in faster drug release, although not as prominently. It has been shown before that gamma irradiation could decrease the water contact angle of electrospun PCL matrices and increase wetting due to the formation of surface polar groups (Augustine et al., 2015). Also, differences in polymer crystallinity noted with solid state analysis can play their part.

The primary mechanism of PCL degradation is hydrolysis of ester groups, which occurs preferentially in the amorphous regions of the polymer where ester bonds are more exposed due to loose structural packing (Bosworth and Downes, 2010). Thus, increased crystallinity could prolong PCL biodegradation and this trend was observed with gamma sterilized PCL and PCL/CAM matrices, although it was not statistically significant. Likewise, Cottam et al. saw that gamma irradiation significantly decreased the rate of PCL degradation (Cottam et al., 2009). Another parameter that could affect the rate of PCL biodegradation is its hydrophobicity/hydrophilicity, as absorption of water and hydration of the polymer chains is the first step necessary for hydrolytic degradation (Bosworth and Downes, 2010). Accordingly, it could be expected that increased water absorption and hydrophilicity would enhance degradation and result in increased loss of mass. Most significant increase in swelling occurred with plasma treated PCL and PCL/CAM matrices, but no change was seen in the loss of mass. As PCL degradation is a long process, one week may have been too short time to fully appreciate the extent of these effects. These longer effects may be more important if tissue scaffolds or implants are designed and less so in case of wound dressings, which need to be changed before differences in degradation become apparent. However, for environmental considerations, faster degradation may also be beneficial.

It could be speculated that with some treatments first the drug on the fiber surface would be degraded (or the drug in fibers on the matrix surface), which could change the burst release profile. In case of PCL/CAM fibers, it was seen that wetting limits the drug release rate. If the drug on the surface of the matrix would be degraded more compared to that inside the fibers, the extent of burst release should be reduced. This is seen in UV-treated mats. UV is known to be surface sterilization method. Although the loss of drug during 30 min UV treatment was not statistically significant in PCL/CAM matrices, it could have been enough to lower the extent of burst release. At the same time, if sterilization/disinfection process would induce interactions between carrier polymers and the drug (e.g. formation of hydrogen bonding), the burst release could also be reduced, but this is not supported by solid state analyses in our study.

Sterilization effects on drug release are affected by both matrix material and drug properties, but also formulation geometry as shown by several studies (Maggi et al., 2004; Petersen et al., 2013). Ethylene oxide sterilization significantly increased sirolimus and atorvastatin burst release from PLLA-coated stents (Petersen et al., 2013), however, Mikkonen et al. demonstrated that ethylene oxide treatment slowed down simvastatin elution from PCL-coated stents (Mikkonen et al., 2009). They also reported that as different drugs have different elution profiles from the same polymeric matrix due to different drug properties, they are also differently affected by gamma sterilization (Mikkonen et al., 2009).

Our results show that in addition to the sterilization efficacy also other important properties of drug-loaded electrospun fiber matrices need to be considered and tested in order to choose the most suitable sterilization/disinfection technique. Summary of the studied effects of different sterilization methods on the investigated electrospun fiber matrices can be found in Table 5.

5. Conclusions

Different techniques enable effective sterilization of electrospun fiber matrices: gamma irradiation (50 kGy dose), UV irradiation for 30 and 60 min on both sides, *in situ* generated chlorine gas treatment (1 h and 2 h). When drug-loaded electrospun fiber matrices are sterilized, the drug degradation as well as polymer changes in fiber matrices need to be separately investigated. It was seen that different carrier polymers were able to protect the drug from degradation differently and thus not only drug susceptibility to degradation needs to be considered. Effects of sterilization on relevant physicochemical and mechanical properties of electrospun drug-loaded and non-loaded fiber matrices were revealed. Although no changes in the morphology were seen, the most crucial was the effect of sterilization on the polymer crystallinity which also is directly changing the mechanical properties and polymer degradation behavior. Some sterilization methods, like argon plasma treatment in our study, can have a tremendous effect on the surface properties of electrospun fiber matrices, affecting wettability, swelling and drug release behavior. Sterilization effects are affected by both matrix material and drug properties, but also formulation geometry. No single sterilization method can be considered appropriate for all

materials and formulations thus case-by-case approach needs to be taken when developing a novel electrospun drug delivery system as a drug product.

Declaration of Competing Interest

The authors declare no conflict of interest.

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Appendix A. Supplementary data

Differential scanning calorimetry thermograms (Figs. S1 and S2) and texture analysis results (Table S1).

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ijpharm.2019.118450.

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