THE EFFECT OF MOLECULAR WEIGHT OF POLYCAPROLACTONE IN BLENDED NANOFIBERS ON THE VAGINAL MICROBIOME RESPONSE

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Abstract

The health of the vaginal microbiota is a crucial component of an intricate system. Vaginal drug delivery systems play an integral role in women's health by offering targeted and efficient treatment options for various gynaecological conditions, while aiming to minimally impact said bacterial population. We propose using nanofibrous materials based on the polymers polycaprolactone (PCL) and poly(lactic acid) (PLA) as a drug delivery system for vaginal environment due to their favourable properties. The molecular weight of PCL can significantly influence the properties of blend nanofibers, which in turn affects their interaction with the vaginal microbiome. The molecular weight of polymers tends to enhance the mechanical properties, crystallinity and stability of the nanofibers, potentially leading to a more controlled and sustained release of incorporated substances. This controlled release is crucial for maintaining a balanced microbiome, as it may help in the release of antimicrobial agents or probiotics.

A healthy vaginal microbiome is dominated by various types of *Lactobacilli*, which produce lactic acid and antimicrobial compounds. We tested the effect of blend nanofibers with PCL of two molecular weights (Mn 45,000 and 80,000) on the growth rate of three Lactobacilli species: *L. crispatus, L. gasseri, and L. jensenii*. There was no distinguishable effect on the growth rates of *L. crispatus* and *L. gasseri*, but the growth rate of *L. jensenii* was affected by PCL of both molecular weights. This is an important finding for the future use of PCL based nanofibers in vaginal drug delivery.

Keywords: Nanofibers, vaginal, polycaprolactone, microbiome, *Lactobacillus*

1. INTRODUCTION

Material composition of drug delivery systems used in vaginal drug delivery is crucial for ensuring effective and targeted treatment. They need to be biocompatible to avoid causing irritation or adverse reactions, and their properties must support the controlled release of medications at the site of action. Moreover, these materials help maintain an optimal environment for the natural microbiota, which is essential for overall vaginal health. Currently, vaginal drug delivery employs a variety of dosage forms, each tailored to enhance efficacy and user comfort. These include creams, gels, suppositories, tablets, rings, and films. Each form has unique benefits. For example, vaginal rings offer prolonged and controlled drug release, while gels and creams can provide immediate relief. On the other hand, many of these formulations still have disadvantages due to specifics of the vaginal environment. This includes its self-cleaning properties, acidic environment and most importantly vaginal bacterial population, which plays a crucial role in this intricately balanced system. Nanofibers are emerging as a promising system for vaginal drug delivery due to their unique properties [1], and so their testing for interaction with vaginal microbiota is essential.

Lactobacillus crispatus, *Lactobacillus jensenii*, and *Lactobacillus gasseri* are key players in maintaining vaginal health in Caucasian and Hispanic population [2,3]. These beneficial bacteria help maintain an acidic pH, which

prevents the overgrowth of harmful microorganisms. They produce lactic acid and hydrogen peroxide, both of which are natural antimicrobials. Additionally, these lactobacilli enhance the mucosal immune response, providing further physiological protection against infections. Each novel material intended for vaginal use must be tested for interaction with these bacteria in order to assess its biocompatibility.

Polycaprolactone (PCL) and poly(lactic acid) (PLA) nanofibers have been widely researched for drug delivery applications due to their biocompatibility and biodegradability [4,6]. The molecular weight of polymeric chains combined with fiber dimeter can impact the bulk mechanical properties of the resulting nanofibers. Higher molecular weight PCL was described to produce nanofibers with increased mechanical strength, crystallinity and slower degradation kinetics, which is beneficial for long-term drug delivery applications. On the other hand, lower molecular weight PCL can result in nanofibers with faster degradation rates, suitable for applications requiring quicker drug release [7]. Lower molecular weight also increases frequency of the chain end groups affecting surface functionality, reactivity and energy, thereby affecting interactions between nanofibers surface and biological environment [8]. The choice of molecular weight depends on the specific therapeutic needs and desired release kinetics. As there is a wide range of potential vaginal application, it is crucial to test the response of vaginal microbiota for both higher and lower molecular weight PCL. For this study, polycaprolactone with molecular weight of 45,000 and 80,000 Da was chosen. Nanofibers were produced by blend electrospinning of the PCL and PLA mixture, tested for their basic morphological and surface properties and then their effect on growth kinetics of three vaginal bacterial strains was assessed for 44 hours. Presence of adhered bacteria was evaluated after the incubation period by electron microscopy.

2. MATERIALS AND METHODS

2.1 Materials

The polymers used for nanofibers preparation were biodegradable polyesters poly(lactic) acid (PLA, Corbion, Purac Biochem bv, NL) and poly-ε-caprolactone (PCL, Sigma-Aldrich, Mw 45,000 and 80,000 Da). The ratio of the polymers in the final spinning solution was 50:50 (wt.). Nanofibrous sheets were prepared from chloroform-based solvent system while utilizing DC needleless electrospinning device Nanospider™ (NS Line 1WS500U, Elmarco) set to production speed 15 mm/min.

2.2 Morphological and wettability analysis

Morphology of the nanofibers was evaluated by scanning electron microscopy (Tescan Vega 3 SEM). The fiber diameters were measured using built in software and expressed as histograms of diameter distribution and mean ± standard deviation. The initial water contact angle of sessile drop was measured on Drop Shape Analyzer DSA30E (Krüss, software 1.16 DSA30) in 5 repeats per sample. Contact angle development was measured after 1, 3 and 5 minutes on selected sample.

2.3 Interaction with vaginal *Lactobacilli*

Lactobacillus strains were *Lactobacillus jensenii* (CCM 7560), *Lactobacillus gasseri* (CCM 7009) and *Lactobacillus crispatus* (CCM 7776). Prior to the experiment, each lactobacilli strain underwent three passages during the exponential growth phase to ensure optimal metabolic activity. All three strains were incubated in De Man, Rogosa and Sharpe broth (MRSB, HiMedia), supplemented with 0.1% L-cysteine (Merck) as a reducing agent, under microaerophilic conditions at 37°C with CO_2 tension.

Materials used for testing interactions with vaginal lactobacilli were cut into 1 cm diameter circles and sterilized using UV light. For the experiment, each sample was immersed into 3.9 ml of bacterial suspension of MRSB and 0.1ml of the third passage of the bacterial strain. Optical density at 600 nm (OD600) was measured at specified intervals (0, 2, 4, 6, 8, 12, 17, 19, 21, 24, 32, and 44 hours) to assess turbidity. An increase in OD600 value reflects a rise in bacterial cell concentration. After 44 hours of incubation, the materials were gently

washed with sterile phosphate buffered saline and fixed with 2.5 % paraformaldehyde for 10 minutes. The materials were then assessed by SEM for the presence of adhered bacteria.

3. RESULTS AND DISCUSSION

In this work, two types of biodegradable nanofibrous materials comtaining 50:50 mixture of two polymers – PLA and PCL. The difference between the materials was given by variation in molecular weight of PCL, which was 45,000 and 80,000. Combination of PLA and PCL represent a potentially new material for use in gynaecological applications, and as such, the response of vaginal microbiome to different PCL component needs to be assessed.

The materials were firstly evaluated by SEM for fiber diameter distribution. The PLA/PCL45 fibers had smaller mean fiber diameter (0.76 ± 0.3 µm) compared to the PLA/PCL80 nanofibers. Both showed randomly oriented fibrous structure with smooth surface of nanofibers (**Figure 1**).

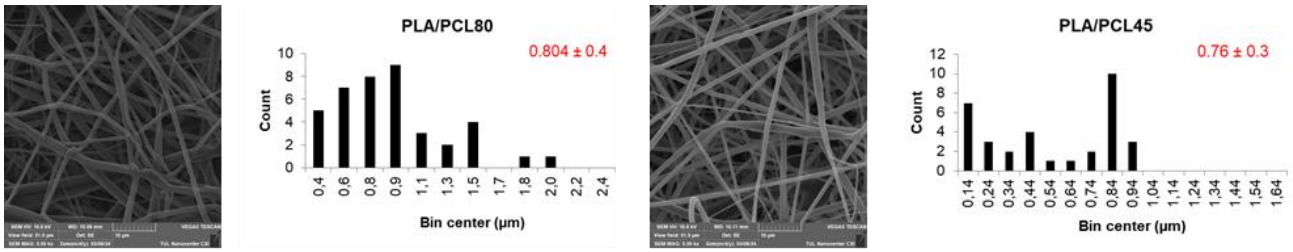


Figure 1 Scanning electron microscope images and histograms of the measured fibers size distribution including mean ± standard deviation values (red).

There was no significant difference between the samples observed with the sessile water drop wettability measurement. Based on the results, both of the materials can be considered hydrophobic, as the initial contact angle (CA) over 90° (**Figure 2a**). Accelerated wettability in time was observed for the PLA/PCL80. The contact angle dropped to 85.26° after 5 minutes while the PLA/PCL45 remained more hydrophobic with CA(m) 96.85° (**Figure 2b**).

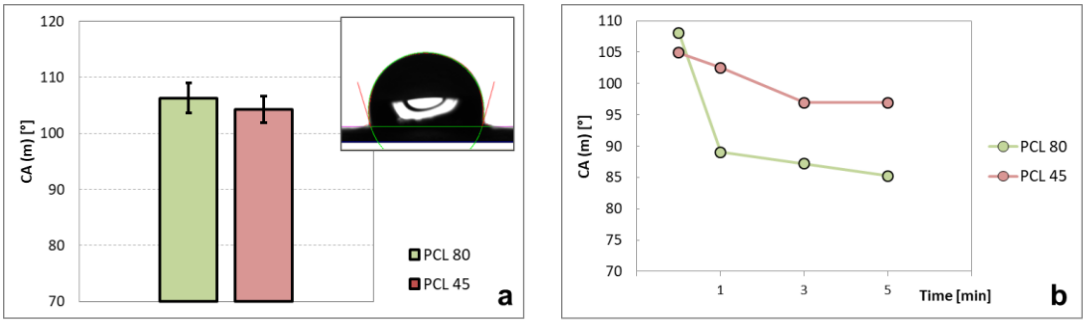


Figure 2 Contact angle of PLA/PCL80 and PLA/PCL45 materials (labeled by their PCL component), a) initial contact angle, b) contact angle after 1, 3 and 5 minutes.

The growth of three Lactobacilli strains was monitored by measuring optical density at a wavelength of 600 nm, where light scattering is referred to as a factor of turbidity, rather than light absorption. Growth of bacterial cells typically progresses through a series of consecutive phases including lag, log and stationary. When plotted, the OD600 data of *L. crispatus*, *L. jensenii* and *L. gasseri* from 44 hours of incubation (**Figure 3**) shows that *L. jensenii* growth curve is flatter indicating slower bacterial cells proliferation. Lactobacilli generally as a genus are considered aerotolerant anaerobes that produce lactic acid through the fermentation of glucose [9]. However, *L. jensenii* is considered to be an anaerobic strain [10], and so the experiment condition which were

microaerophilic, were suboptimal for its growth. Nevertheless, the growth kinetics was the same in three consecutive experiments, so it was considered as a normal growth for this type of bacteria.

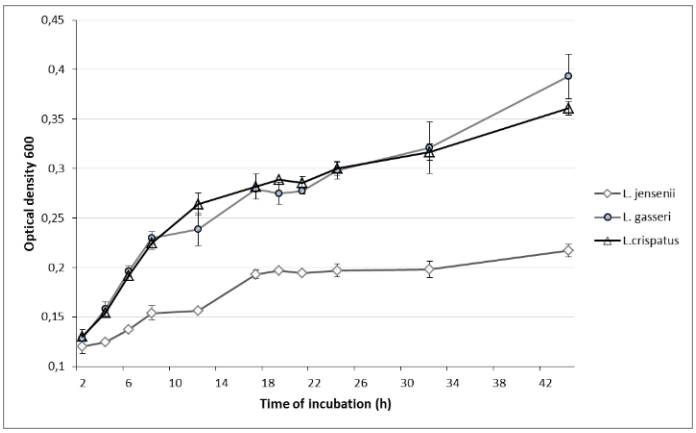


Figure 3 The growth curves of *Lactobacillus crispatus, Lactobacillus jensenii and Lactobacillus gasseri* incubated for 44 hours under microaerophilic conditions at 37 °C.

Coincubation with PLA/PCL materials yielded similar results with *L. crispatus* and *L. gasseri*. There is no difference in growth kinetics with or without contact with either of the tested nanofibers (**Figure 4**).

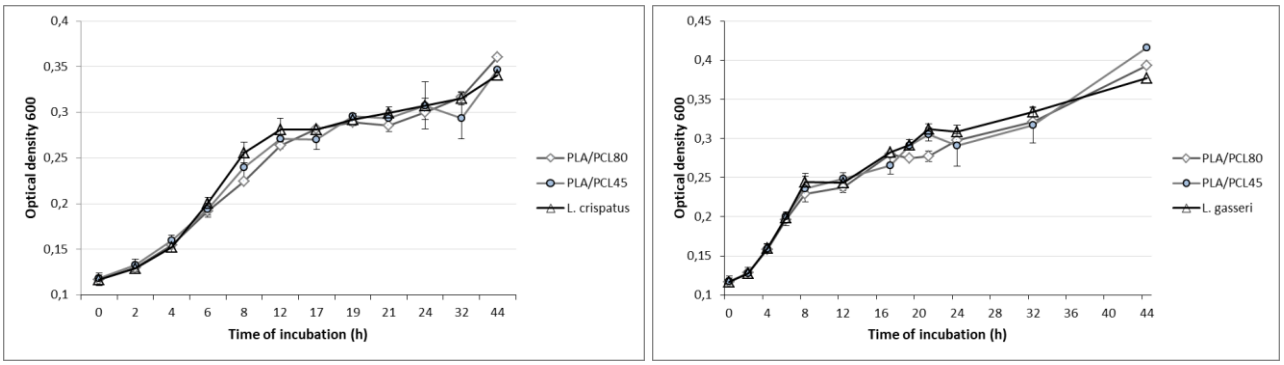


Figure 4 The growth curves of *Lactobacillus crispatus (left) and Lactobacillus jensenii (right)* in contact with tested materials PLA/PCL$_{80}$ and PLA/PCL$_{45}$. Incubation for 44 hours under microaerophilic conditions at 37 °C.

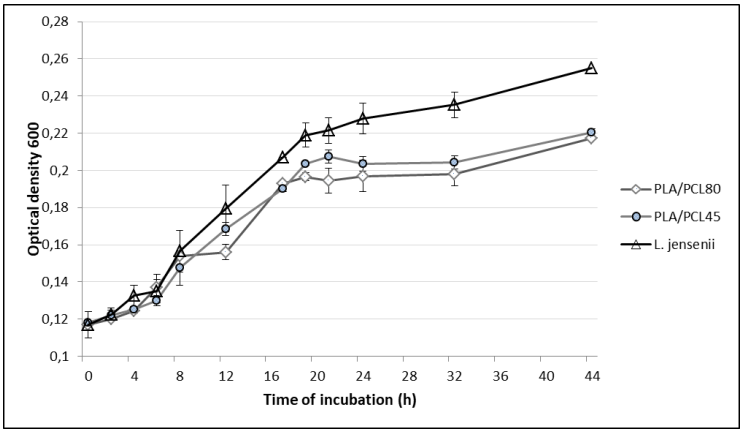


Figure 5 The growth curves of *Lactobacillus gasseri* in contact with tested materials PLA/PCL$_{80}$ and PLA/PCL$_{45}$. Incubation for 44 hours under microaerophilic conditions at 37 °C.

Coincubation of PLA/PCL materials with *Lactobacillus jensenii* resulted in different growth curve compared to positive control (bacterial suspension without any material) (**Figure 5**). The bacteria coincubated with the PLA/PCL materials demonstrated slower growth rate after 20 hours of incubation and reached lower maximum optical density after 44 hours. There is no difference between growth kinetics of *L. jensenii* in contact with either PLA/PCL80 and PLA/PCL45.

The materials were assessed by scanning electron microscopy after coincubation with bacteria (**Figure 6**). There are very few bacteria attached to the fibers after 44 hours, with the exception of PLA/PCL45 and *Lactobacillus jensenii*, which exhibited strings of rod-shaped bacterial cells on its whole surface serving as an evidence of increased adhesion of *L. jensenii* to the surface of PLA/PCL45 nanofibers. This could contribute to *L. jensenii* altered growth in presence of this material, nevertheless, it does not explain the similar trend in PLA/PCL80 material, which also altered the growth without the bacterial adhesion.

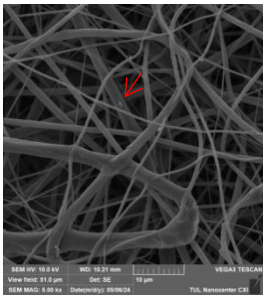
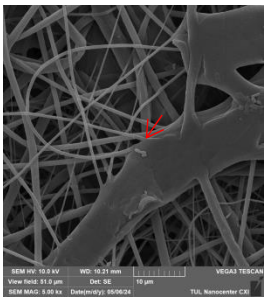
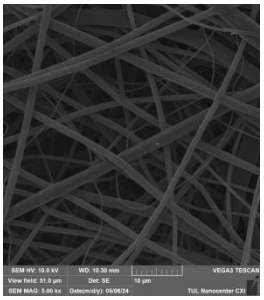
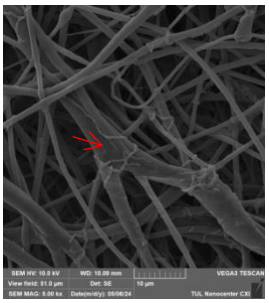
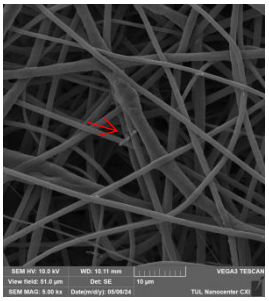
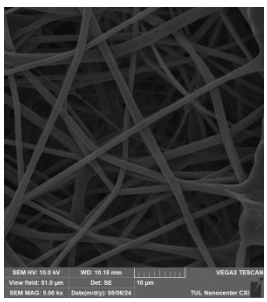
Material	***L. jensenii***	***L. gasseri***	***L. crispatus***
PLA/PCL_{80}			
PLA/PCL_{45}			

Figure 6 SEM images of PLA/PCL80 and PLA/PCL45 nanofibers after 44 hours (microaerophilic conditions, 37 °C) of coincubation with vaginal bacteria. Red arrows indicate bacterial cells attached to the fibers.

4. CONCLUSION

The objective of this study was to evaluate potential impact of molecular weight of PCL in blend PLA/PCL nanofibers on the growth of three vaginal bacterial strains: *Lactobacillus crispatus, Lactobacillus jensenii,* and *Lactobacillus gasseri*. The lower molecular weight (45.000 Da) of PCL resulted in nanofibres with smaller average diameter while maintaining hydrophobicity after 5 minutes (CA 96.95°). The incorporation of lower molecular weight PCL also led to increased adhesion of *Lactobacillus jensenii* with a growth curve comparable to sample containing higher molecular weight PCL (PLA/PCL80). Growth curves of *Lactobacillus crispatus* and *Lactobacillus gasseri* remained unaffected by any of the tested samples.

The obtained results indicate potential of the PLA/PCL nanofibres for applications in vaginal environment stimulation and drug delivery. Further research on biodegradation and biocompatibility is needed.

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