Electrospun Nanofibers for Dual Probiotic and Antibiotic Delivery Targeting Bacterial Vaginosis

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Abstract

Introduction: Bacterial vaginosis (BV) is characterized by replacement of vaginal *Lactobacilli* with other anaerobes. Metronidazole (MET) is the standard treatment, but recurrence rates exceed 50%. *Lactobacillus* administration helps microbiome recolonization, but frequent administration impacts patient compliance. This study evaluates novel PEO/PLGA electrospun nanofibers as vehicles for dual delivery of MET and sustained *L. crispatus*, detailing the fiber properties and their ability to both deplete anaerobes and release antimicrobial agents.

Methods: Nanofibers used a 3:1 ratio of PEO to PLGA. 50 µg of MET and 4.42x10^7 CFUs of *L. crispatus* were loaded into the fiber. 20 kV were applied to the PEO and PLGA solutions with extrusion rates of 0.3 mL/hr and 0.1 mL/hr, respectively. PEO and PLGA syringes were horizontally opposite, 15 cm and 18 cm from a central mandrel, respectively (Fig. 1A). The mandrel was spun at 200 rpm to generate a mesh architecture. Nanofibers were characterized using SEM imaging and thermogravimetric analysis. *L. crispatus* recovery over one week in MRS broth and MET release into simulated vaginal fluid (SVF) over one hour were measured. Nanofiber inhibition on the growth of *Gardnerella* during coculturing was assessed using serial dilutions.

Results: SEM imaging displayed meshed nanofiber architecture (Fig. 2). A maximum of 78% of the theoretical MET load was released within 20 minutes of nanofiber immersion in SVF (119.25 µg/mL, Fig. 4A). Release of *L. crispatus* from dual-loaded fibers was consistent over 6 days for probiotic-only and dual-loaded antibiotic-probiotic fibers, with a total cumulative release of 1.29x10^9 CFUs/mg of fiber (Fig. 4B). Release profiles demonstrated a burst release of MET within the first hour of fiber incubation, followed by sustained release of probiotic *L. crispatus*. *Gardnerella* was eliminated within 24 hours in co-cultures with *L.* crispatus, MET-loaded fibers, and dual-loaded fibers.

Conclusion: The similar probiotic release from dual-loaded and *L. crispatus*-only fibers supports the feasibility of dual antibiotic and probiotic delivery. Results suggest that nanofibers may act as a novel delivery vehicle for both MET and *L. crispatus* in treating BV, with the burst of antibiotic aiming for clearance of pathogens and the sustained probiotic release aiming to restore a lactobacilli-dominant microflora.

Nanofiber Formulation

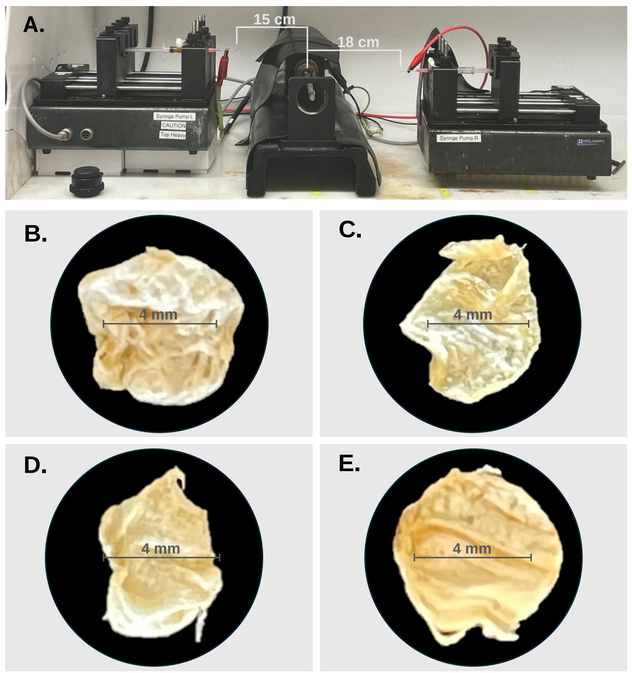

Figure 1. A) Image of the electrospinning apparatus. 250 mg of PEO was co-spun in a 3:1 ratio with 5% w/w PLGA. 20 kV were applied at the needle tips, and fiber was collected on a rotating mandrel. Metronidazole and *L. crispatus* were added to the PEO mixture before spinning. Metronidazole was dissolved in dimethylformamide, and *L. crispatus* was suspended in 500 µL of MRS. B) Blank nanofiber segment. C) Metronidazole-only nanofiber segment. D) *L. crispatus*-only nanofiber segment. E) Dual-loaded metronidazole-*L. crispatus* nanofiber segment.

Scanning Electron Microscopy

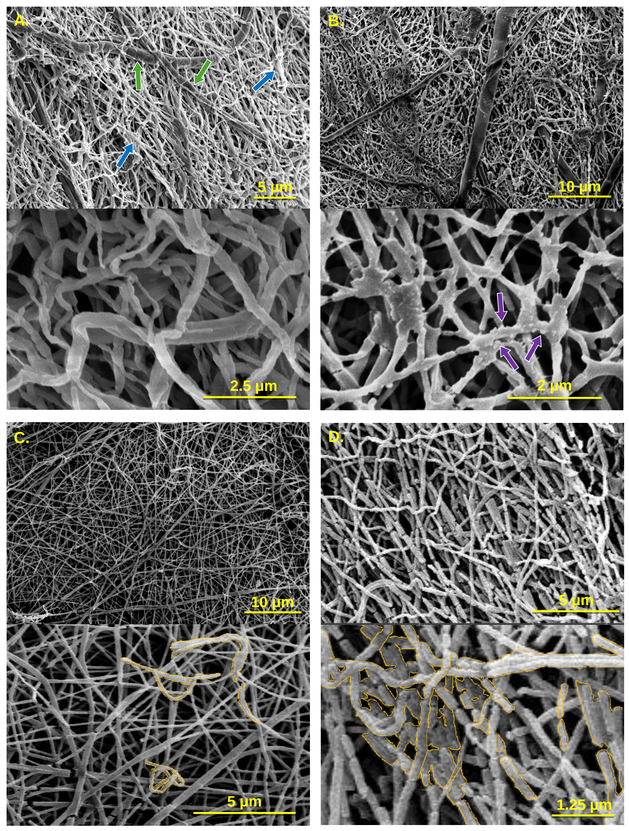

Figure 2. SEM images of electrospun nanofibers. Top images show the architecture of the fibers. Bottom images are further magnified for detail.
A) Blank nanofibers. The top image illustrates the PEO/PLGA meshed network. Blue arrows indicate PEO fibers, and green arrows indicate PLGA fibers.
B) Metronidazole-only fibers loaded with 19.2 µg of metronidazole per mg of fiber. Purple arrows in the bottom image indicate crystals of metronidazole incorporated into the PEO fiber strands.
C) *L. crispatus*-only fiber loaded with 4.42x10^7 CFUs of probiotic. Incorporated *L. crispatus* is highlighted in orange in the magnified image.
D) Dual-loaded fiber with metronidazole and *L. crispatus*. *L. crispatus* is highlighted in orange in the bottom image. Due to the quantity of *L. crispatus*, metronidazole is not visible.

Thermographic Analysis

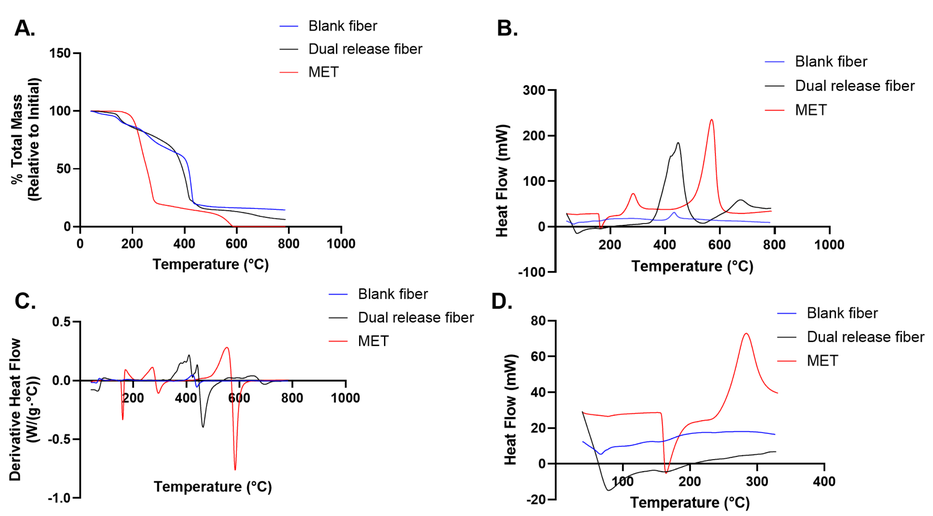

Figure 3. DSC/TGA characterization. A) TGA thermogram. The rapid decline in mass corresponds to the thermal decomposition point of metronidazole (200°C). The mass losses for the PEO/PLGA fibers also match expectations. B) DSC thermogram of metronidazole, blank fibers, and dual release fibers. Conformation to expected thermographic patterns confirms fiber composition. C) Derivative DSC. D) Closer image of the DSC thermogram from B. The initial endothermic dip for metronidazole corresponds to the thermal decomposition point in the TGA (Fig. 3A). The shift in this dip for the dual-loaded fiber also closely matches the leftward shift in peaks seen in B.

Metronidazole and *L. crispatus* Release

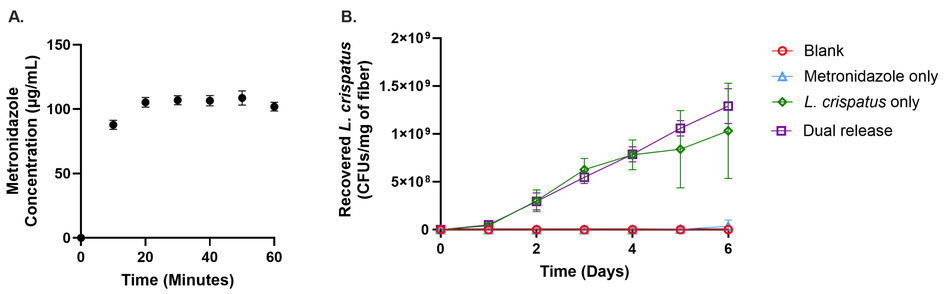

Figure 4. A) Mean and standard deviation of metronidazole concentration in supernatant after incubating 8 mg of nanofibers in SVF for the stated time (n=3), determined via absorption at 320 nm. Release represented up to 78% of the theoretical load. B) Cumulative release of *L. crispatus* from 15 mg of PEO/PLGA nanofibers over 6 days (n=5). Determined by serial dilutions on MRS plates with daily PBS washes.

Fiber Degradation

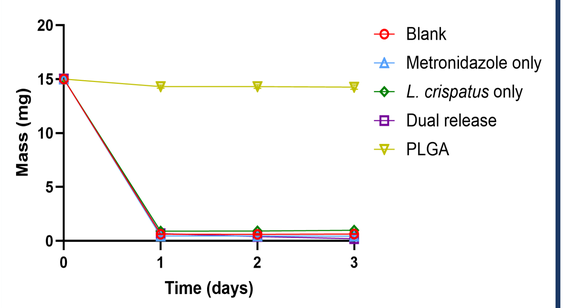

Figure 5. Change in mass for 15 mg fiber segments in 1 mL of SVF over 3 days (n=3). Fibers were incubated at 37°C for 24 hours, then left to dry for another 24 hours.

Figure 5 (cont.). Fiber mass was then taken, and the fibers were re-immersed in SVF.

The substantial drop in mass after the initial time point was likely the complete dissolution of PEO. Small PLGA flakes that the PEO helped secure were also lost.

Mass loss for the fully PLGA fiber was minimal, as expected based on its extreme hydrophobicity.

Quantification of Live *L. crispatus*

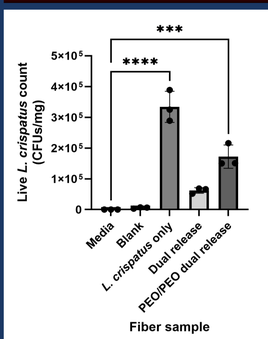

Figure 6. Quantification of viable bacterial titers in fibers immediately after the electrospinning process. Fibers were composed entirely of PEO to enable full dissolution in Amies transport medium. To assess the effects of the PLGA solvent, an additional fiber of dual-loaded PEO in MRS co-spun with PEO in 1,1,1,3,3,3-Hexafluoro-2-propanol (PLGA solvent) was used (PEO/PEO dual release). Fibers were partitioned into 15 mg segments and fully dissolved. Bacteria were live/dead stained with SYTO 9/PI and quantified using a MiraPro Microbial Cell Counter.

Inhibition of *Gardnerella* in a Coculture

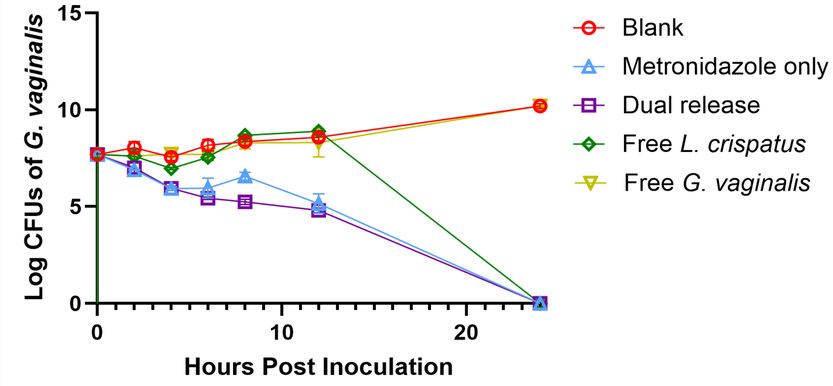

Figure 7. Log CFUs of *Gardnerella vaginalis* over 24 hours when co-cultured with nanofibers in NYC III media. Initial *Gardnerella* load was 5x10^7 CFUs/mL. Free *L. crispatus* was loaded at 3x10^7 CFUs/mL to reflect the theoretical load in the *L. crispatus*-laden fiber segments. At 12 hours, a 3-log drop in *Gardnerella* titers was observed for metronidazole-loaded and dual-loaded fibers. *Gardnerella* was eliminated with metronidazole-fibers, free planktonic *L. crispatus*, and dual release fibers after 24 hours. Separate plating on MRS agar demonstrated viable growth of *L. crispatus* from the planktonic and dual release supernatants.

Conclusions & Future Studies

- PEO/PLGA electrospun nanofibers provide a treatment modality for the simultaneous and temporally independent delivery of metronidazole and *L. crispatus* to target bacterial vaginosis with the goal to reduce recurrence.
- Therapeutically relevant loads of up to 74.53 µg of metronidazole/mg of fiber were delivered in a burst release within the first minute of fiber exposure to SVF.
- Sustained *L. crispatus* release was observed over 6 days with a cumulative recovery of up to 1.33x10^9 CFUs/mg of fiber. Recovery was similar for probiotic-only and dual-loaded fibers.
- *Gardnerella* titers were reduced within 4 hours of exposure to metronidazole-loaded fibers, and elimination was observed within 24 hours for metronidazole-loaded fibers, free probiotic, and dual-loaded fibers.

Future Work will perform competition and preventive assays to assess the efficacy of dual delivery electrospun nanofibers at reducing vaginal pathogen burden and restoring a lactobacilli-dominant vaginal microenvironment *in vivo*.

Acknowledgements

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