Development of MET-Loaded EVOH/CHI Nanofibers to Prevent Endometriosis

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Abstract:

Endometriosis is a debilitating condition characterized by the growth of uterine lining, or endometrium, outside of the uterus. Its underlying causes remain unclear, resulting in delayed diagnosis and limited treatment options. Emerging evidence has linked endometriosis to Fusobacterium, a bacterium common in the oral cavity. [1] In mice, treatment with the antibiotic metronidazole (MET) has been shown to slow endometriotic progression. [2] However, MET delivery presents several challenges. Oral, cream, or gel-based formulations result in off-target effects and low drug retention, emphasizing the need for a new delivery platform. This work focuses on designing a MET-loaded nanofiber capable of sustainably and locally delivering MET to the uterus to prevent the spread of endometriosis. Poly (ethylene vinyl alcohol), known as EVOH, and chitosan (CHI) nanofibers loaded with MET were fabricated using electrospinning. Nanofibers were analyzed with scanning electron microscopy (SEM) and energy dispersive X-ray (EDX). The drug release profile and cytotoxic effects of nanofibers and free MET were evaluated in vitro.

Summary of Research:

Nanofiber Synthesis: EVOH and CHI nanofibers were prepared following protocol and parameters in Sasaki et. al. (2024) to obtain electrospinning solutions with final CHI concentrations of 0.4% and 0.7% [3]. MET was incorporated in predetermined final concentrations: 1.25%, 0.625%, and 0.3125% by weight of polymer content. Prior to observation, samples were coated with platinum. Nanofiber morphology was observed with FE-SEM. Nanofiber composition was estimated with EDX.

As seen in Figure 1, electrospun nanofibers exhibited relatively uniform morphology. The EVOH nanofiber without MET or CHI had bead formation inconsistent with previous studies, potentially due to issues with the electrospinning machine. Otherwise, observed fiber morphology is consistent with studies on similar materials. EDX demonstrated compositional changes

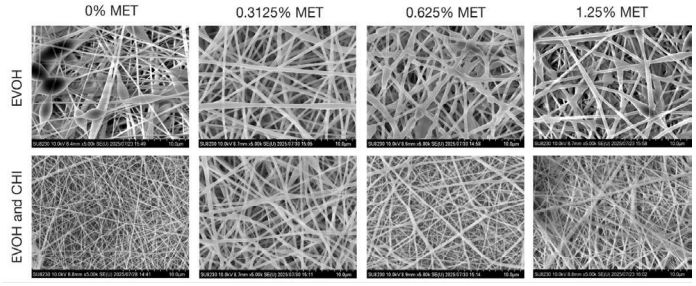

Figure 1: SEM image of thin film. Deposition conditions:RF 75W, Ar 10 sccm, 45 minite deposition time.

in each formulation, indicating MET and CHI were successfully incorporated into the nanofibers.

Drug Release Study: PBS (pH 6.9) and acetate buffer (pH 4.5), both with 0.5% Tween-80, were used in the drug release study. 1 cm^2 of each nanofiber was placed into 10 mL of solution in a vial. Vials were stirred and incubated at 37 °C, with 5 mL samples being replaced with fresh buffer intermittently. Each sample was compared to a standard curve of MET concentration in

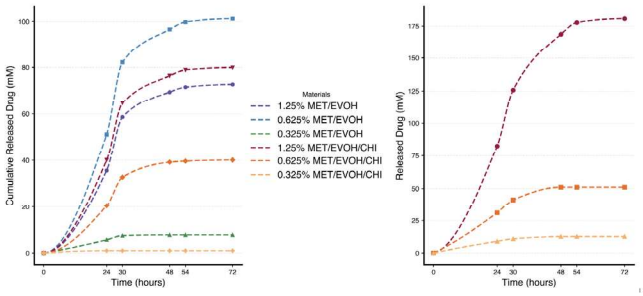

Figure 2:Temperature dependence of impendence against frequency. Deposition conditions: RF 50W, Ar 8.5 sccm, O2:1.5 sccm, Deposition time 4 hours.

the buffers using UV-VIS.

A sustained release of MET for 72 hours was demonstrated for all nanofibers, as seen in Figure 3. In neutral conditions, 1.25% MET/EVOH, 0.625% MET/EVOH, and 1.25% MET/EVOH/CHI nanofibers had a cumulative release of MET above 50 mM, indicating potential cytotoxicity. CHI-based nanofibers had a greater release of MET in acidic conditions than in

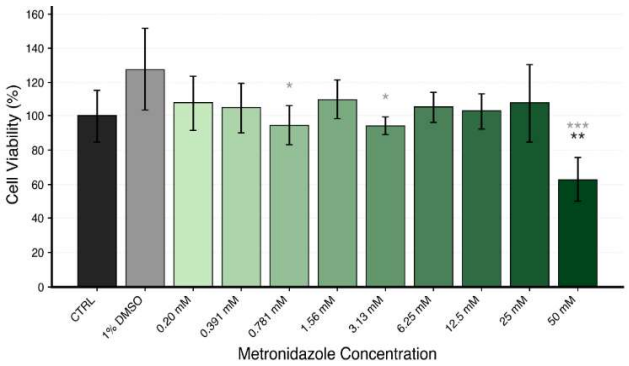

Figure 3: 2D Schematic of Nanoionic Device

neutral conditions, demonstrating pH-responsivity.

Cell Studies: Human endometrial (12Z-EEC) cells were seeded at $5x10^4$ cells per well in a 96-well plate for 6 hours. 0.5 cm^2 nanofibers were sterilized with EtO. Free MET and nanofibers were dissolved with 1% DMSO in vitro. The MTT Cell Viability Assay was conducted by precipitating formazan for four hours and solubilizing overnight before measurement. Dunnett's test was completed for each study. A p-value less than 0.001 was represented by ***, less than 0.01 by **, and less than 0.05 by *.

Figure 3 displays the viability of 12Z-EECs treated with free MET in varied concentrations. There was a significant decrease in viability for the 50 mM treatment group compared to both the negative and positive controls. These findings suggest 50 mM of MET is approximately the lethal concentration for 50% of 12Z-EEC cells (LC50) after 24 hours.

The percent viability of cells treated with synthesized nanofibers is represented in Figure 4. There was a notable increase in cell viability for the 0.325% MET/EVOH, 0.625% MET/EVOH/CHI, and 1.25% MET/EVOH/CHI nanofibers when compared to the negative control. Cells treated with 0.625% MET/EVOH and 1.25% MET/EVOH nanofibers had a significant decrease in viability compared to the positive control. This may be due to an influx of MET greater than its LC50 as demonstrated in the drug release experiment. 1.25% MET/EVOH/CHI nanofibers, which also showed release above the LC50, had greater cell viability than the negative control. This may be related to CHI's biological properties or a decrease in MET release without Tween-80.

Conclusion and Future Steps:

MET-loaded nanofibers were successfully synthesized, as demonstrated by SEM and EDX. Abnormal bead formation in the EVOH control nanofiber indicates synthesis should be repeated with a stronger electrospinning machine. Likewise, investigating higher MET and CHI concentrations is an area of interest. As EDX is limited for organic molecules, nanofibers should be further characterized via Fourier-transform-IR (FTIR), thermal analyses (TGA, DSC), and SEM image analysis to confirm present functional groups, weight % composition, and nanofiber morphology, respectively. Additional analysis on properties of interest, such as hydrophobicity and mucoadhesion should be investigated.

Preliminary drug release data demonstrated measurable release over 72 hours for all nanofibers. A longer drug release study should be conducted with a decreased amount of surfactant, if any, to assess physiological release more closely.

MET exhibits an LC_{50} around 50 mM in 12Z-EEC cells after 24 hours. This study should be repeated at concentrations between 25 mM and 100 mM of MET to fully encapsulate cytotoxicity. CHI-based MET nanofibers showed no cytotoxicity to endometrial cells after 24 hours. Cell viability with free MET and nanofibers should be assessed in vitro for 14 days, the traditional duration of an antibiotic regimen. Additional assessments should include conducting a scratch assay

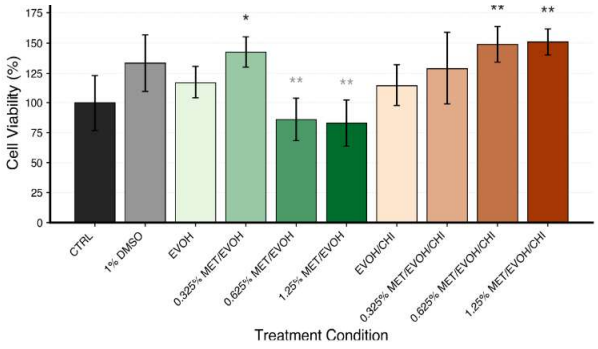

Figure 4: Transistor gate voltage sweep in vacuum.

within a fusobacteria/human endometrial cell co-culture to mimic biological endometriosis progression.

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References:

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