Supplementary Materials

Electrospun Fibres of Chitosan / PVP for the effective chemotherapeutic drug delivery of 5-Fluorouracil

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Table S1: Concentrations of PVP and CS in specific solvent systems along with the electrospinning results obtained.

	Label	Solvent	Result/Comment
8% PVP		TFA only	Some droplets on foil. Forms Taylor cone. One (and rarely 2) fibre jets. Fibres formed
8% PVP 8% CS	-	TFA:Acetic Acid 9:1	No fibre formation.
6% PVP 6% CS	-	TFA: Acetic Acid 9:1	No fibre formation.
6% PVP 5% CS	-	TFA: Acetic Acid 9:1	No fibre formation.
6% PVP 4% CS	E	TFA: Acetic Acid 9:1	Fibre formation.
6% PVP 3% CS	D	TFA: Acetic Acid 9:1	Fibre formation.
6% PVP 2% CS	C	TFA: Acetic Acid 9:1	Fibre formation.
6% PVP 1% CS	B	TFA: Acetic Acid 9:1	Fibre formation.
6% PVP 0% CS	A	TFA: Acetic Acid 9:1	Fibre formation.

Table S2: instrumentation parameters that have been tested to produce robust electrospun fibres.

Solution	Voltage	Tip-Collector Gap	Flow Rate	Result
6% PVP 4% CS	10kV	10 cm	1 ml/hr^{-1}	No fibre formation. Voltage too low to form Taylor Cone.
6% PVP 4% CS	20kV	10 cm	1 ml/hr^{-1}	No fibre formation. Voltage too high. Taylor Cone distorted.
6% PVP 4% CS	15kV	10cm	1 ml/hr^{1}	No fibre formation.
6% PVP 4% CS	15kV	15cm	1 ml/hr^{1}	Fibres partially forming. Flow rate too high.
6% PVP 4% CS	15kV	15cm	0.5ml/hr^{1}	Fibres partially forming. Flow rate too high.
6% PVP 4% CS	15kV	15cm	0.1mL/hr^{-1}	Fibre formation.

Drug Release:

Instrumentation set-up:

Samples were analysed using a PerkinElmer UV/VIS Spectrometer (Lambda 35). UV Win Lab software was programmed with a full scan set-up, starting at 700nm and ending at 200nm with an ordinate mode of 1nm slit width. Scan speed was set at 480nm/nm with a data interval of 1 nm. 1 cycle with a cycle time of 1 second was used.

Experimental analysis:

A standard curve consisting of free 5-Fu in PBS (pH 7.4) was created using 3.5µg/mL, 7.5µg/mL, 15µg/mL and 30µg/ml solutions. The line equation of $y = 0.0304x + 0.0297$ with an R^2 value of 0.9994 was taken from the standard curve for the determination of the concentration of 5-Fu released from electrospun scaffolds (x denotes the λ_{max} value while y is the 5-Fu concentration). The wavelength, λ_{max}, at which a substance has its strongest photon absorption of 5-Fu, was determined at approximately 266nm.

Electrospun samples were cut into approximately 1cm x 1cm squares. Three samples from each mat (1 mg/mL, 5 mg/mL and 10 mg/mL 5-Fu mats) were then immersed in 4mL of PBS solution (pH 7.4) in a 12 well plate which was then covered in aluminium foil and placed into an incubator at 37°C with horizontal rotating shaking (at 80 rpm). After 3, 24 and 48 hours, 0.4 mL of well contents was taken and added to 3.6mL of fresh PBS (1 in 10 dilution) to read on the UV-VIS spectrometer. 0.4mL of fresh PBS was then added back into each well. The absorbances read from the instrument were multiplied by 10 to adjust for the dilution factor. The concentration of 5-Fu released from each sample was calculated using the line equation from above.

Drug release results:

Figure S1 highlights the 5-Fu released from each sample (1 mg/mL, 5 mg/mL and 10 mg/mL) over 3, 24 and 48 hours.

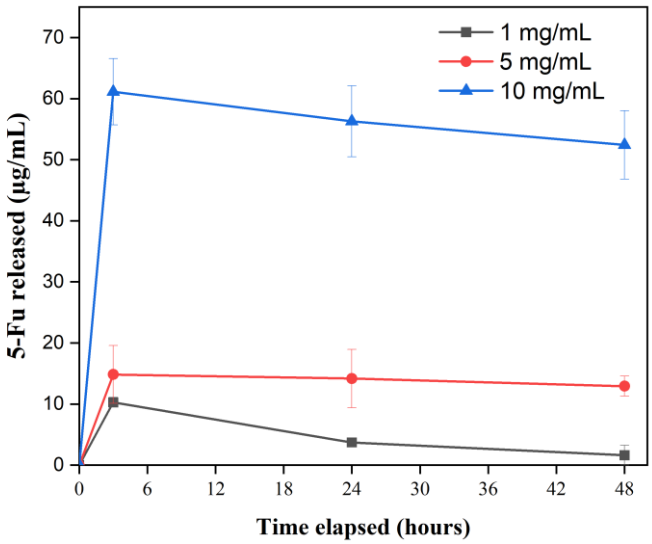

Figure S1. 5-Fu released (µg/mL) from samples 1 mg/mL, 5 mg/mL and 10 mg/mL at 3, 24- and 48-hour time points.

This data was then plotted as a cumulative release for further analysis of the results (see Figure S2). The 1 mg/mL sample cumulatively released 10.27 µg/mL, 13.98 µg/mL and 15.60 µg/mL of drug over 3, 24 and 48 hours (Figure S2). From Figures S1 and S2, it is suggested the sample underwent a "burst" release after 3 hours. This "burst" profile, which is commonly associated with drug release from nanofibres, relates to the migration of the drug to the fibre surface [1]. However, it may also apply to drugs on or near the surface to begin with. In our case, due to 5-Fu being hydrophilic, this would

contribute to a burst release type of profile. Phase separation along the surface of the fibres can also contribute to this phenomenon [2].

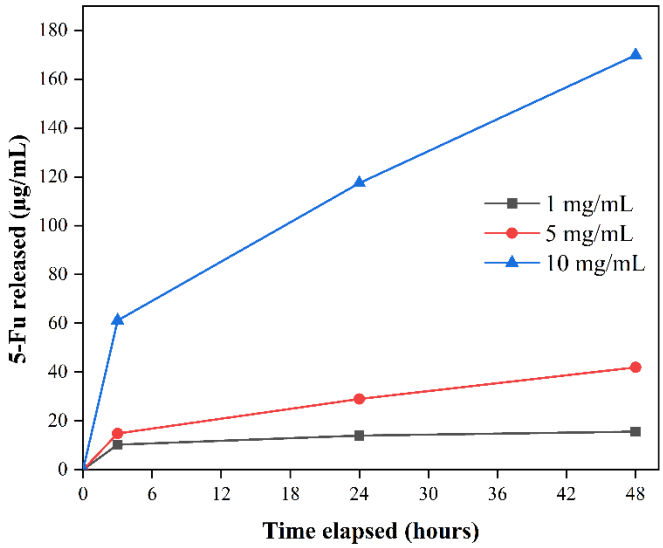

Figure S2. Cumulative release of 5-Fu (µg/mL) from samples 1 mg/mL, 5 mg/mL and 10 mg/mL 5-Fu after 3, 24 and 48 hours.

The 5 mg/mL sample saw a cumulative drug release of 14.83 µg/mL, 29.00 µg/mL and 41.93 µg/mL over 3, 24 and 48 hours, respectively. The 10 mg/mL sample cumulatively released 61.12 µg/mL, 117.41 µg/mL and 169.85 µg/mL of drug after 3, 24 and 48 hours, respectively. Both samples conformed to the initial 3-hour "burst" release seen in the 1 mg/mL sample. Burst release occurs when an initial large bolus of drug is released immediately upon placement into the release media, before the release rate reaches a stable profile [3]. This quick mechanism of 5-Fu release can relate to the drug moving from a superficial area of fibres using both diffusion and dissolution mode [4].

As the exposure time of the scaffolds in PBS increased to both 24 and 48 hours, a more prolonged, sustained release was evident (Figures 1 and 2), particularly relating to samples 5 mg/mL and 10 mg/mL. This may be due to dissolution of the fibrous surface, resulting in diffusion of the drug from the inside of the material [4, 5]. Zhang *et al.*, 2014, have presented similar results showing an initial "burst" of 5-Fu from an electrospun composite containing polylactide nanofibres, followed by a more controlled release over time [6]. Undesirable side effects of administering pristine anti-cancer drugs along with the poor half-lives usually associated with these drugs would be effectively negated through the construction of a prolonged, controlled 5-Fu releasing vehicle [7].

A single factor ANOVA test of the 3 hours group saw a significant variation within the means of samples 1 mg/mL, 5 mg/mL and 10 mg/mL (P=0.000742, n=3, $P < 0.05$ denotes statistical significance). Post hoc t-tests (two-sample, assuming unequal variances) showed that there was statistical differences between the 1 mg/mL and the 10 mg/mL groups (P = 0.01705), along with the 5 mg/mL and 10 mg/mL groups (P= 0.006306, n=3) (P< 0.05 denotes statistical difference). This ANOVA and *t*-test analysis were performed for both the 24 hours groups and 48 hours groups. Regarding the 24 hours groups, ANOVA analysis again showed variation within the means of the samples (P= 0.000969, n=3). With this, two-sample *t*-tests showed statistical differences between the 1 mg/mL and the 10 mg/mL groups (P = 0.017931, n=3) and the 5 mg/mL and 10 mg/mL groups (P= 0.0102, n=3). Similarly, under the same test conditions, a significant variance within the means of each group were found in the 48 hours groups (P= 0.000295, n=3). Statistical differences were seen between the 1 mg/mL and 10 mg/mL groups (P = 0.0177, n=3) and the 5 mg/mL and 10 mg/mL groups (P= 0.0310, n=3), following post hoc t-tests.

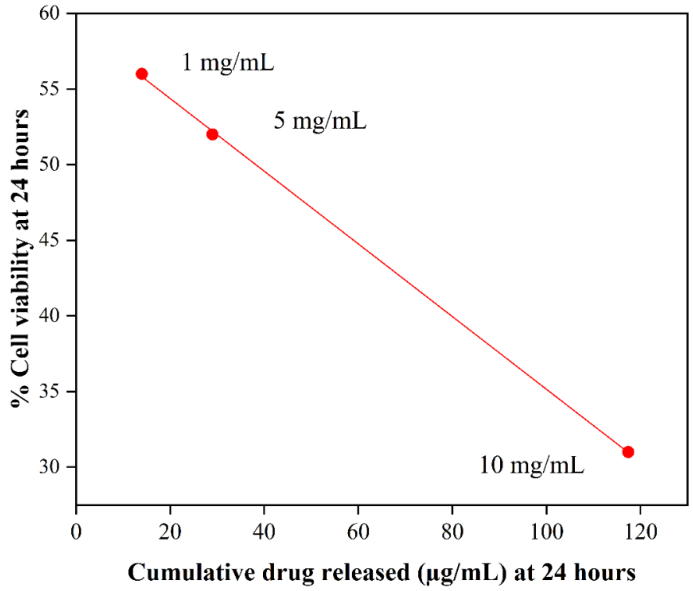

Figure S3. Cumulative drug release (µg/mL) vs % cell viability at each concentration (0, 1, 5, 10 mg/mL).

At each time point, the drug released from the 1 mg/mL sample was statistically different to only the 10 mg/mL sample. The drug released from the 5 mg/mL sample was statistically differently to only the 10 mg/mL sample at each time point also. These results correlate strongly to the cell viability assays where similar statistical significance results occurred. The 1 mg/mL sample was not efficient at releasing significant concentrations of 5-Fu to effectively kill A549 cells over 24 hours. There is a negative correlation between cumulative drug release at 24 hours for each concentration of drug and cell viability at 24 hours (Figure 3) (Pearson's r = -0.99989).

As per the correlation between drug release and cell viability, concentrations initially loaded with 5 mg/mL and 10 mg/mL of 5-Fu are sufficient at releasing enough drug over 24 hours to effectively kill A549 cells. Results also suggest that following an initial "burst" release after 3 hours, there is still drug being released from the samples over 24 and 48 hours (Figures 1 and 2) [7, 8]. This may benefit potential future studies where samples with 5-Fu in the range of 5 mg/mL to 10 mg/mL will be investigated for their potential as a sustained-release drug delivery system for *in-vivo* models [9, 10]. Now that the efficacy of the drug release system has been shown, it is planned to study the efficiency of the system in delivering drug over a longer period.

Initial studies showed non-significant degradation of samples over two weeks in saline conditions at 37°C. Future work will also assess long-term degradation, under both physiological and stressed conditions.

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