Supplementary information

A multiphasic core-shell flurbiprofen and ciprofloxacin-loaded nanofibrous dressing cures infected burns via dual control of infection and inflammatory response

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Physicochemical characterization of dressing

Fourier transform infrared spectroscopy (FTIR)

FTIR-ATR spectroscopy (Thermo Scientific IS50) was performed by using diamond crystal ATR for assessing the chemical structure of nanofibrous wound dressing. Spectra were recorded in the range of 400-4000/cm at 32 scans and scan speed 1cm/s. The intensity was measured in terms of % transmittance with a resolution of wavenumber in 4 cm-1. Following the acquisition, Originlab software was used to annotate and analyze the ATR-FTIR spectra. Nanofibrous wound dressing with and without drug is compared with control from these spectra.

Morphology

Field-emission scanning electron microscopy (FESEM) (JEOL JSM-7610F Plus, JEOL Ltd., Japan) was used to visualize the morphology of the fabricated dressing. The prepared nanofibrous dressing was firstly sputtered with gold to make it conductive and visualized in FESEM. The porosity of fabricated dressing and fiber diameter were investigated at different magnifications. Moreover, the average diameter of fibers was calculated using ImageJ software (NIH, USA) using FESEM images.

The Co-axial Structure

The core-shell structure of nanofibers was determined by HR-TEM (Themis 300G3). For this, a carbon-coated copper grid was placed on a drum collector, and for a few seconds, nanofibers were directly deposited onto it. Further, it was dried and observed in HR-TEM. Further, the image was processed by using the software ImageJ.

Hydrophilicity

The hydrophilic property of a dressing is determined by measuring the contact angle with deionized water at room temperature. The contact angle of dressings was measured by the sessile drop method using Holmarc's Contact Angle Meter. The sample of 10 mm × 10 mm on a glass slide was placed on the stage of this instrument and a drop of 5 µL DI water was automatically dispersed onto the sample and the image was captured with a Charged Coupled Device (CCD) camera fitted in this instrument. ImageJ software was used to calculate the contact angle from this image and the values are mean ± standard deviation (n=3).

Swelling potential

The swelling ability of dressing was obtained by immersing the sample in PBS (pH=7.4) and calculating swollen weight at various time points. The sample of 5mm×5mm (wt=10mg) was immersed in PBS (pH=7.4) and incubated at 37^{o}C (n=3)[1]. The sample was collected at predetermined time points, wiped with tissue paper to clean the surface, and swelling potential was calculated using this equation:

$$\text{Swelling capacity } \% = ((Ws - Wi)/Wi)\ [\![x100]\!]\text{^}$$

Where Ws is the swollen weight and Wi is the initial weight of each sample respectively.

In-vitro degradation measurement

Enzymatic degradation was studied wherein the sample of 5 mm×5 mm (wt=10 mg) was incubated in collagenase type I (0.05 mg/ml) in TESCA buffer at 37^{o}C at gentle shaking [2]. At different time points, the enzyme solution was discarded, and the sample was dried and weighed. Three samples were used for each time point.

$$weight\ loss\ \% = ((Wi - Wd)/Wd)\ [\![x100]\!]\text{^}$$

Cell viability and proliferation assay

NHDF was passage when reached at 70-80% confluency. Further, the NHDF was a subculture in Dulbecco's Modified Eagle Medium (DMEM) (Gibco) adding 10% FBS and 1% A-A solution. The dressing was cut into 5mm diameters and sterilized with 70% ethanol wash followed by irradiation with UV light for 30 min on each side. Samples were washed with PBS at pH 7.4 twice in 96 cell culture well plate. The adhered NHDF in the flask were firstly trypsinized with trypsin-EDTA solution 1X (Himedia) for 3-4 min and centrifuged to obtain a cell pellet. Cell suspension was obtained and seeded onto each dressing sample (n=3) with a cell population of 2×10^{4} cells/well. The cell culture plate (TCP) was used as a control group. Three sets of plates seeded for 1, 3, and 7 days. The media was changed after every 3rd day. At a predefined time, 10 µL of 5 mg mL^{-1} of 3-[4, 5-dimethylthiazol-2-yl]-2, 5-diphenyl tetrazolium bromide (MTT) solution was added to each well followed by covering with aluminum foil. After incubation of 4hr in an incubator, the supernatant was discarded and 100 µL of dimethyl sulfoxide solution (DMSO) was added to each well to dissolve formazan crystal. Strong agitation for 15 min in a plate reader, followed by a read of absorbance at 570 nm in a UV-Vis Spectrophotometer (Clariostar microplate reader) was taken.

Effect on histological architecture of the burn wound

Hematoxylin and eosin staining protocol [1]

- Deparaffinization: Paraffin is removed from tissue sections using xylene.
- Rehydration: Tissues are rehydrated through a series of graded alcohols (2–3 min each), followed by a 3-min dip in water.
- Hematoxylin Staining: Stain tissues in hematoxylin for 3–4 min.
- Rinsing: Wash tissues twice in deionized (DI) water for 2 min each.
- Differentiation: Dip in 10% acetic acid in 95% alcohol for 1 min.
- Rinse and Bluing: Rinse in DI water and then wash in tap water.
- Alcohol Dip: Dip tissues in 70% alcohol.
- Eosin Staining: Stain with Eosin Y for 3 min.
- Dehydration: Quickly dip in 90% and then 100% alcohol.
- Clearing and Mounting: Wash in xylene, air-dry, and mount the tissue using D.P.X.

Masson's trichrome staining protocol [2]

- Deparaffinization: Slides with skin tissue are placed in a staining jar and deparaffinized by submerging in three changes of absolute xylene, 4-5 min each.

- Rehydration: Slides are sequentially immersed in descending grades of ethanol: 100%, 95%, 90%, 80%, and 70% ethanol, each for 3-4 min.
- Bouin's Fixation: Slides are submerged in pre-warmed Bouin's solution at 60°C for 30 min to enhance staining.
- Rinsing: Slides are washed under running tap water until the yellow coloration disappears.
- Nuclear Staining: Slides are stained with haematoxylin for 5 min and quick dip in alcohol acidic water and then in ammonia water to visualize nuclei. Further, washed in running tap water for 2 min.
- Cytoplasm Staining: Slides are immersed in Biebrich scarlet red for 5 min.
- Followed by rinsing in tap water.
- Mordant Treatment: Slides are treated with phosphotungitic and phosphormolybdic acid for 10 minutes.
- Collagen and Fibroblast Staining: Without rinsing, slides are immediately transferred to aniline blue solution for 5 minutes.
- Final Wash and Differentiation: Slides are washed in running tap water for 2 minutes, then treated with 1% acetic acid solution for 1 minute.
- Dehydration: Slides are dehydrated in ascending ethanol concentrations: 70%, 80%, 95%, and 100%, each for 1 minute.
- Clearing and Mounting: Slides are cleared in absolute xylene for 1 minute and mounted with DPX and coverslip for microscopic observation.

Van Gieson's Staining [3]

The tissue was firstly stained with Hematoxylin Stain using step mention above. Then, The sections were incubated for 2 minutes in a picrofuchsin solution (1% acid fuchsin in saturated aqueous picric acid). After staining, the sections were dehydrated and mounted using DPX mounting medium. The final staining results showed collagen fibers in pink, nuclei in black, and muscle tissue in yellow.

Results

FTIR Results

Table S1 FTIR results specifying characteristics peaks of major functional present

Sample	Characteristics peaks
PCL	1721 cm^{-1} C=O for PCL
CS	1065 cm^{-1} β–(1–4) glycosidic linkages for CS and 2872 cm^{-1} C–H stretching vibrations for CS
PCL-CS	1165 cm^{-1} symmetrical C-O-C stretching and 1721 cm^{-1} C=O vibration for PCL 2865 cm^{-1} C-H stretching vibration for CS
PCL-CS/CIP	1025 cm^{-1} C-F stretching and 1365 cm^{-1} due to $V_{(asym)}$ COO^- for CIP
PCL-CS/FLB	1418 cm^{-1} C-H deformation of the ring C-C bond for FLB
PCL-CS/FLB-CIP	1165 cm^{-1} symmetric C-O-C stretching and 1722 cm^{-1} by C=O for PCL 2864 cm^{-1} C-H stretching vibrations for CS 1365 cm^{-1} $V_{(asym)}$ COO^- for CIP 1418 cm^{-1} C-H deformation of the ring C-C bond for FLB

Mechanical properties

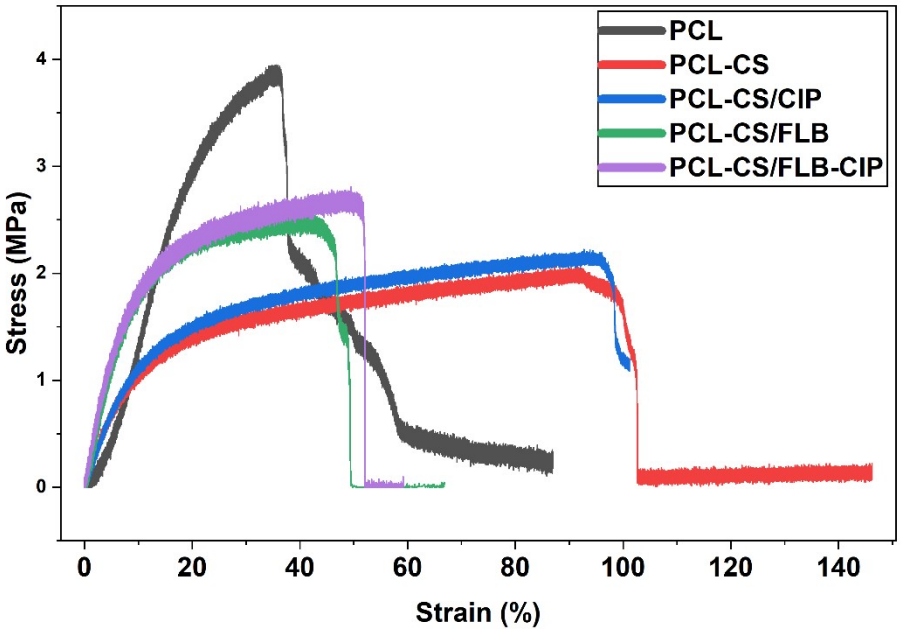

Fig. S1 Stress-strain curve of all dressing

Drug release studies

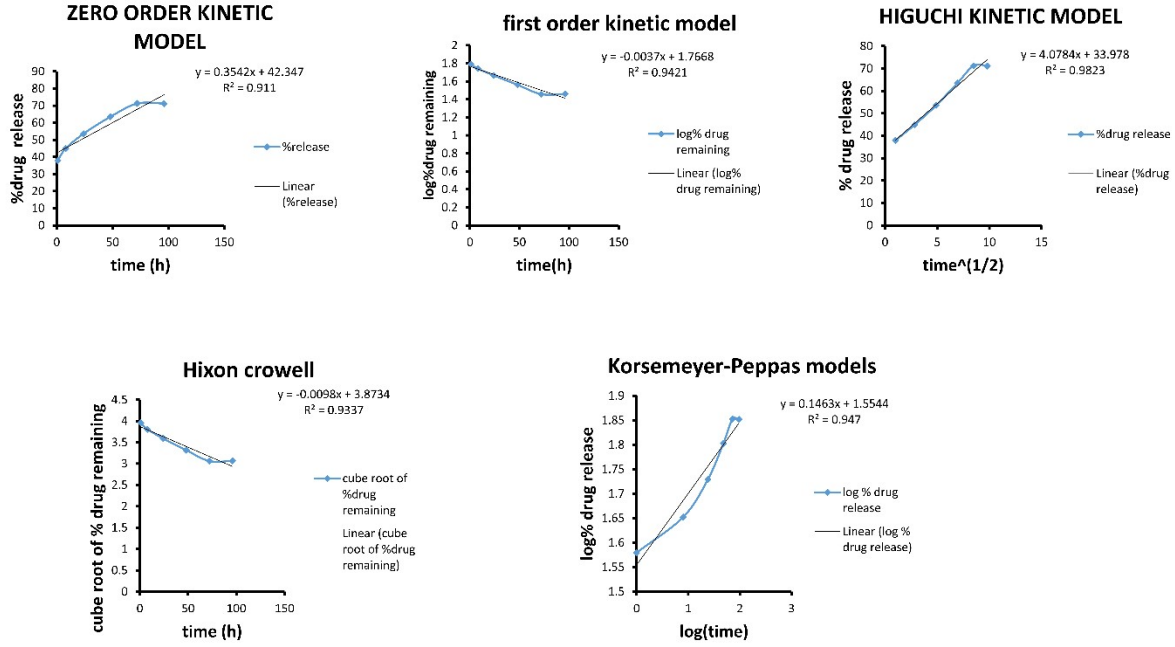

Fig. S2 Release Kinetic models for CIP releases from PCL-CS/FLB-CIP

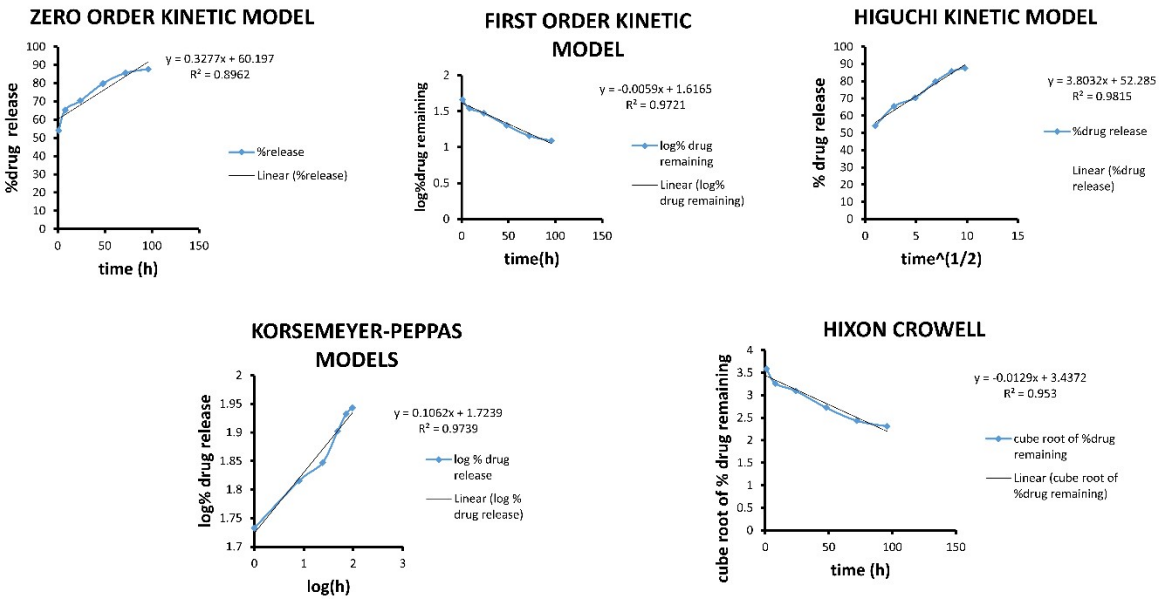

Fig. S3 Release Kinetic models for FLB releases from PCL-CS/FLB-CIP

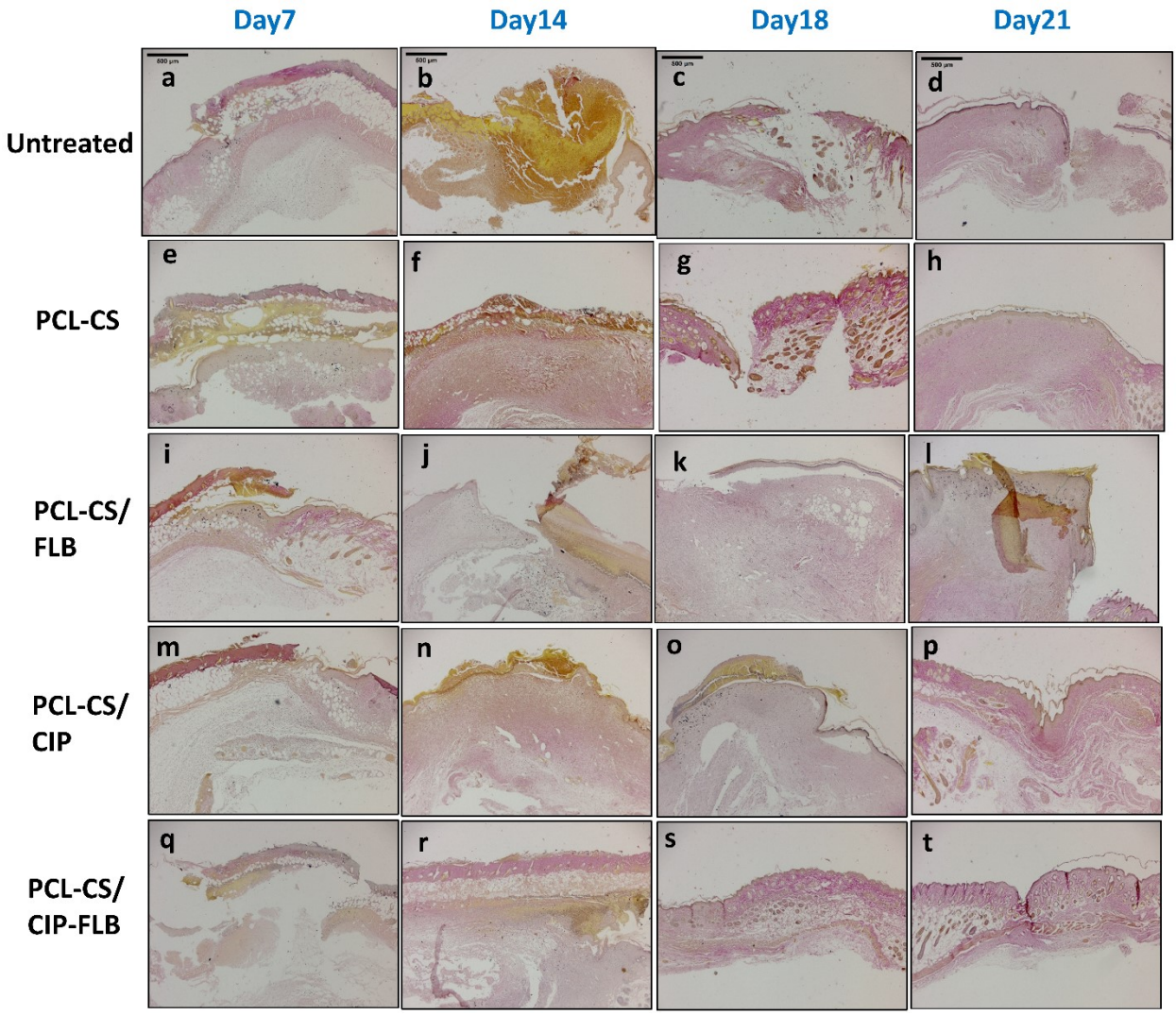

Fig. S4 VG staining of different sample for 7, 14, 18 and 21day (scale bar= 500µm)

References

1. Feldman AT, Wolfe D. Tissue processing and hematoxylin and eosin staining. Methods Mol Biol. 2014;1180:31–43.

2. Assaw S. The use of modified Massion's trichrome staining in collagen evaluation in wound healing study. Malaysian J Vet Res [Internet]. 2012;3(1):39–47. Available from: https://www.researchgate.net/publication/263542834

3. Segnani C, Ippolito C, Antonioli L, Pellegrini C, Blandizzi C, Dolfi A, et al. Histochemical detection of collagen fibers by sirius red/fast green is more sensitive than van gieson or sirius red alone in normal and inflamed rat colon. PLoS One. 2015;10(12):1–10.