Preparation, Characterization and In Vivo Evaluation of Pirfenidone Loaded Layer-by-Layer Films for Dermal Wound Management

P. K. Mandapalli, J. Balija, S. Labala, V. K. Venuganti
BITS Pilani

Purpose
The aim of this study was to investigate pirfenidone (PFD), an anti-fibrotic TGF-beta inhibitor loaded into chitosan (CS) and sodium alginate (SA) layer-by-layer (LbL) films in effective dermal wound management.

Methods
LbL films were prepared by alternative coating of CS (5mg/mL, pH5) and SA (5mg/mL, pH4) onto glass slide template. PFD loading in LbL films is achieved after dissolving it in polymer solution at 10mg/mL concentration before layering. The prepared films and PFD loading were characterized using FTIR, DSC and RP-HPLC. In-vitro release studies of PFD from LbL films were performed in PBS (pH7.4) using Franz diffusion cell apparatus. Cell adhesion study was performed on LbL films using A431 human epidermoid carcinoma cells. In-vivo wound management study was performed on 7 week old female C57/BL6 mice. A circular wound of 8mm diameter was made using a skin biopsy punch. Later, PFD loaded film was topically applied once a day for 12days. Wound contraction was determined by tracing wound area. Tissue samples at wounded area were harvested at time intervals of 0, 3, 6, 9 and 12 days after sacrificing the mice for collagen and total protein estimation. Commercially available povidone-iodine ointment was used as control treatment.

Results
Thickness of the film was found to be 15±2μm as measured by digital micrometer. FTIR and DSC studies confirmed LbL film formation including CS and SA and further confirmed PFD loading in films. PFD loading was found to be 1.0±0.1mg/cm² area of film. In-vitro release studies showed that PFD was released within 3h from LbL films. A431 cells adhesion study showed an average of 3.75x10³ and 4.606 x10³cells/mm² of LbL film adhered to CS and SA layers respectively. In-vivo wound healing studies showed faster (<9days) wound contraction after PFD loaded LbL film application compared to commercial povidone-iodine ointment (12days) and without any treatment (12days). Furthermore, the collagen fraction was significantly (p<0.001) less after 12days with PFD loaded LbL films (5.45±1.07mg/g tissue) compared to povidone-iodine ointment (8.27±0.67mg/g tissue) and without any treatment (39.63±5.39mg/g tissue).

Conclusion
LbL films can be developed as delivery system for dermal wound management. PFD loaded LbL films show potential in effective wound healing.

![Image 1: Effect of topical application of PFD film and Povidone-iodine ointment on collagen content in comparison to control (without any treatment). Data were presented as mean (n=4)±SD. * represents significance between groups at p<0.001.](image1.png)

![Image 2: Representative pictures of wound healing in control, Povidone-iodine ointment and pirfenidone loaded film treated mice on days 0, 3, 6, 9 and 12 post-wounding.](image2.png)