# Electronic Supplementary Information (ESI)

## Dual Crosslinked Hyaluronic Acid Nanofibrous Membranes for Prolonged Prevention of Post-Surgical Peritoneal Adhesion

Chialin Sheu, K.T. Shalumon, Chih-Hao Chen, Chang-Yi Kuo, Yi Teng Fong and Jyh-Ping Chen

### Cytotoxicity of HAFB10 NFM

Cytotoxicity of the NFM was examined according to ISO 10993-5. A HAFB10 NFM (1.5-cm diameter) was extracted in 1 ml of cell culture medium (DMEM supplemented with 10%(v/v) FBS and 1%(v/v) antibiotic-antimycotic) at 37 °C for 1 day, and the extract was collected for cell culture. Fresh culture medium was used as the control. 3T3 fibroblasts were seeded ($1\times10^4$ cells/well) in a 24-well tissue culture plate and cultured with the extract for 4 days at 37 °C in a humidified 5% $CO_2$ environment. Cell viability was monitored at days 1 and 4 by the MTS assay. The absorbance was measured at 492 nm using an ELISA plate reader (BioTek Synergy HT) and normalized to that of the control at each time interval, which was set at 100%.

As can be seen from Figure S1, after normalization to the absorbance from the control experiment (cell culture medium), the relative cell viability in the extraction medium of the HAFB10 NFM was found to be not significantly different from that of the control at both time points. We thus ruled out the cytotoxicity of the HAFB10 NFM.

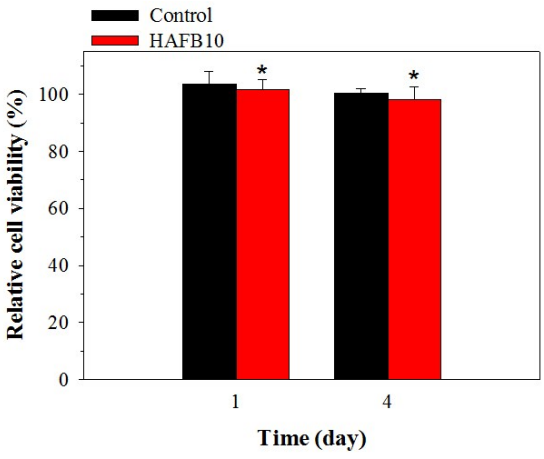

**Figure S1**. Cytotoxicity of HAFB10 NFMs. The cell viability was determined by normalizing with the culture medium control at each time point. *$p > 0.05$ compared with the control. The data are expressed as the means ± standard deviations (n = 6).

***In vivo* biodegradation**

*In vivo* biodegradation was performed on 8 week old Sprague-Dawley rats (SD rats), as per the guidelines of Institutional Animal Care and Use Committee (IACUC) of Chang Gung University. Both HAF30 and HAFB10 were selected for biodegradation *in vivo*, for day 0 and day 7, with three rats at each time point and total of 6. All the rats were anesthetized through intramuscular injection using 300 mg/kg each of 2% Rompun and Zoletil followed by shaving of rat abdomen. The shaved abdomen skin was disinfected with 70% alcohol followed by a 4-cm midline incision using a surgical scalpel blade. HAF30 and HAFB10 membranes (2 cm × 2 cm) were sutured separately on the peritoneal wall, at both sides of midline incision. The abdominal wall and skin of rats were closed using a 4-0 polyamide 6 Ethilon (Ethicon, USA) suture and Biomycin antiseptic ointment was applied on the surgical site. To evaluate *in vivo* biodegradation, the rats were further anesthetized and observed for the membrane appearance at two time points, 0 (2 hours) and 7 days. The abdomen of the rat was opened up through a c-shaped notch, covering both membranes at opposite sides of midline incision. The shape and morphology of each implanted membrane at day 7 was compared with the membrane at day 0 (2 hours).

As shown in Figure S2, the images at day 0 and day 7 indicate both HAF30 and HAFB10 NFMs will degrade *in vivo*, without inducing any chronic effects for surrounding tissues. Similar to *in vitro* degradation, the degradation of HAFB10 is slower than HAF30 from the change in membrane size due to additional crosslinking of the NFM by BDDE.

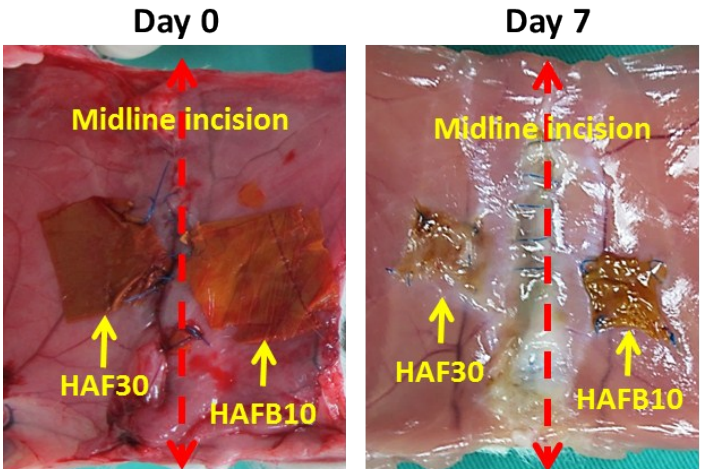


**Figure S2.** Gross view of the *in vivo* implanted HAF30 and HAFB10 NFMs at day 0 and day 7.