

## Supporting Information

### Electrospun pectin-polyhydroxybutyrate nanofibers for retinal tissue engineering

Siew Yin Chan<sup>a, b</sup>, Benjamin Qi Yu Chan<sup>b, c</sup>, Zengping Liu<sup>d</sup>, Bhav Harshad Parikh<sup>d</sup>, Kangyi Zhang<sup>b</sup>, Qianyu Lin<sup>b, c</sup>, Xinyi Su<sup>d, e, f, g</sup>, Dan Kai<sup>b</sup>, Wee Sim Choo<sup>a\*</sup>, David James Young<sup>a, b, h\*</sup>, and Xian Jun Loh<sup>b, c, g\*</sup>

<sup>a</sup> School of Science, Monash University Malaysia, Jalan Lagoon Selatan, 47500 Bandar Sunway, Selangor, Malaysia

<sup>b</sup> Institute of Materials Research and Engineering (IMRE), Agency for Science, Technology and Research (A\*STAR), 2 Fusionopolis Way, Innovis, Singapore 138634, Singapore

<sup>c</sup> Department of Materials Science and Engineering, National University of Singapore, 9 Engineering Drive 1, Singapore 117576, Singapore

<sup>d</sup> Department of Ophthalmology, Yong Loo Lin School of Medicine, National University of Singapore, 1E Kent Ridge Road, Singapore 119228, Singapore

<sup>e</sup> Institute of Molecular and Cell Biology (IMCB), Agency for Science, Technology and Research (A\*STAR), 61 Biopolis Drive, Proteos, Singapore 138673, Singapore

<sup>f</sup> Department of Ophthalmology, National University Hospital, 5 Lower Kent Ridge Road, Singapore 119074, Singapore

<sup>g</sup> Singapore Eye Research Institute (SERI), 11 Third Hospital Avenue, Singapore 168751, Singapore

<sup>h</sup> Faculty of Science, Health, Education and Engineering, University of the Sunshine Coast, Maroochydore DC, Queensland 4558, Australia

**Corresponding authors\* and emails:**

Wee Sim Choo: [choo.wee.sim@monash.edu](mailto:choo.wee.sim@monash.edu)

David James Young: [dyoung1@usc.edu.au](mailto:dyoung1@usc.edu.au)

Xian Jun Loh: [lohxj@imre.a-star.edu.sg](mailto:lohxj@imre.a-star.edu.sg)

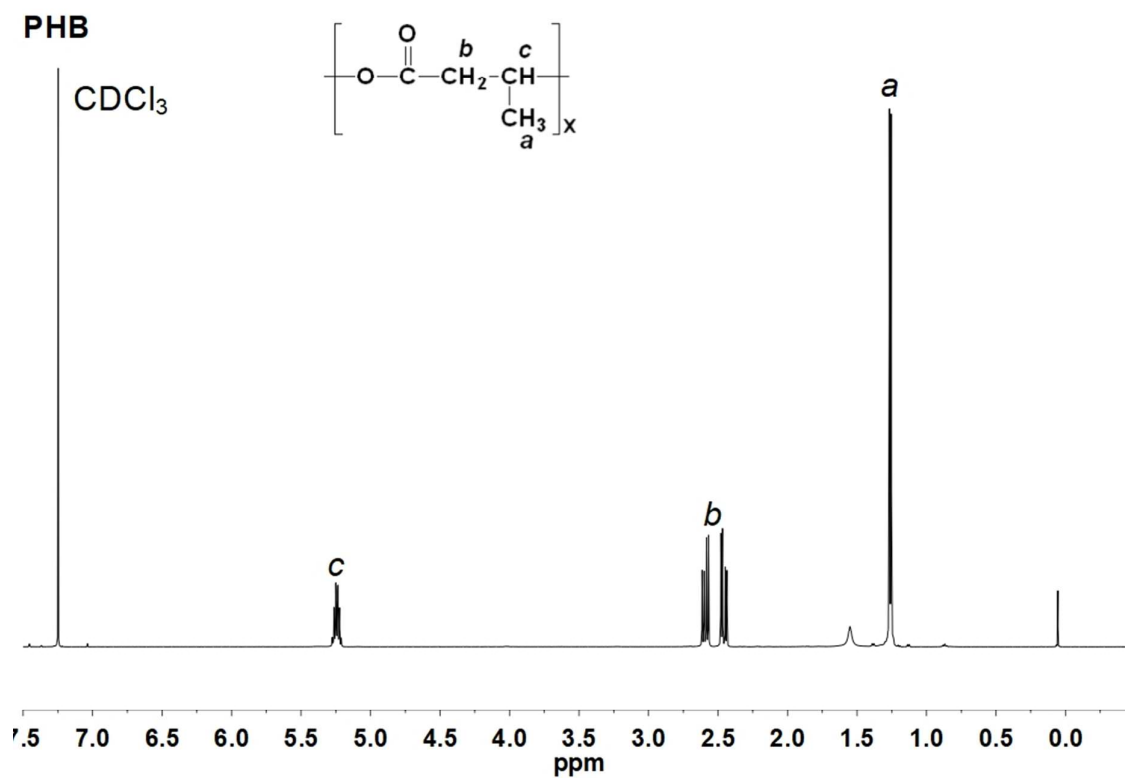


Figure S1. <sup>1</sup>H NMR (500 MHz) spectrum of PHB.

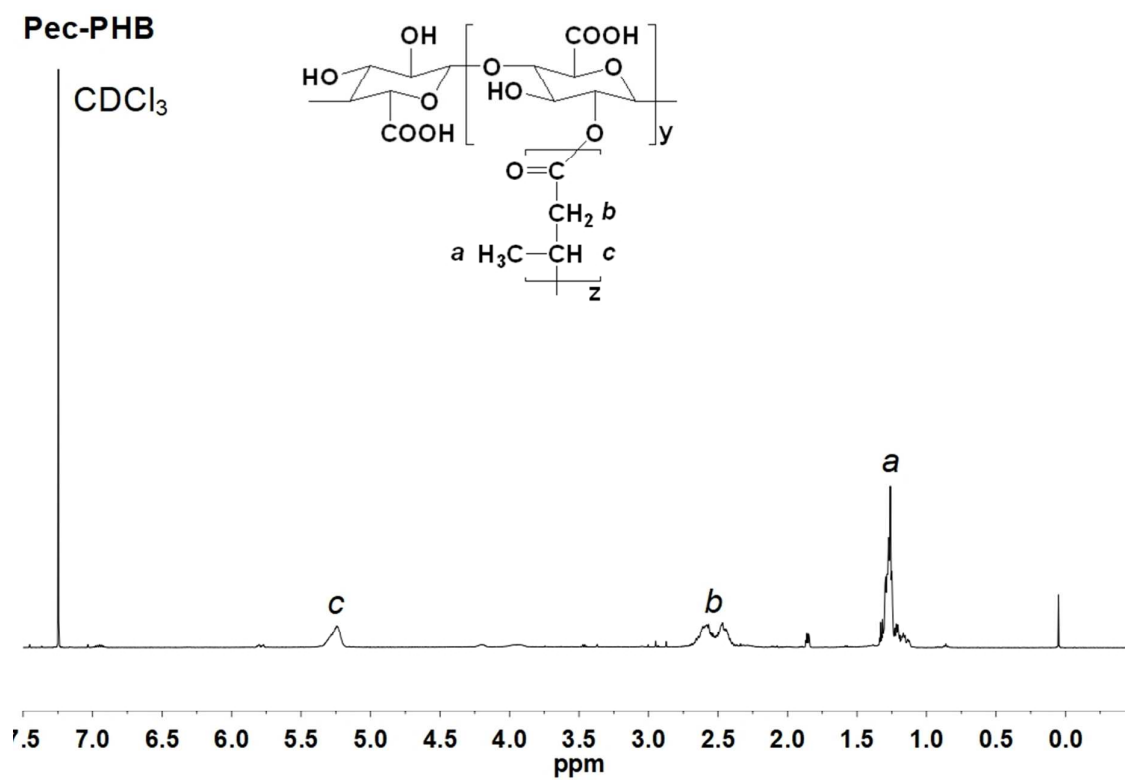


Figure S2. <sup>1</sup>H NMR (500 MHz) spectrum of pec-PHB.

Pectin

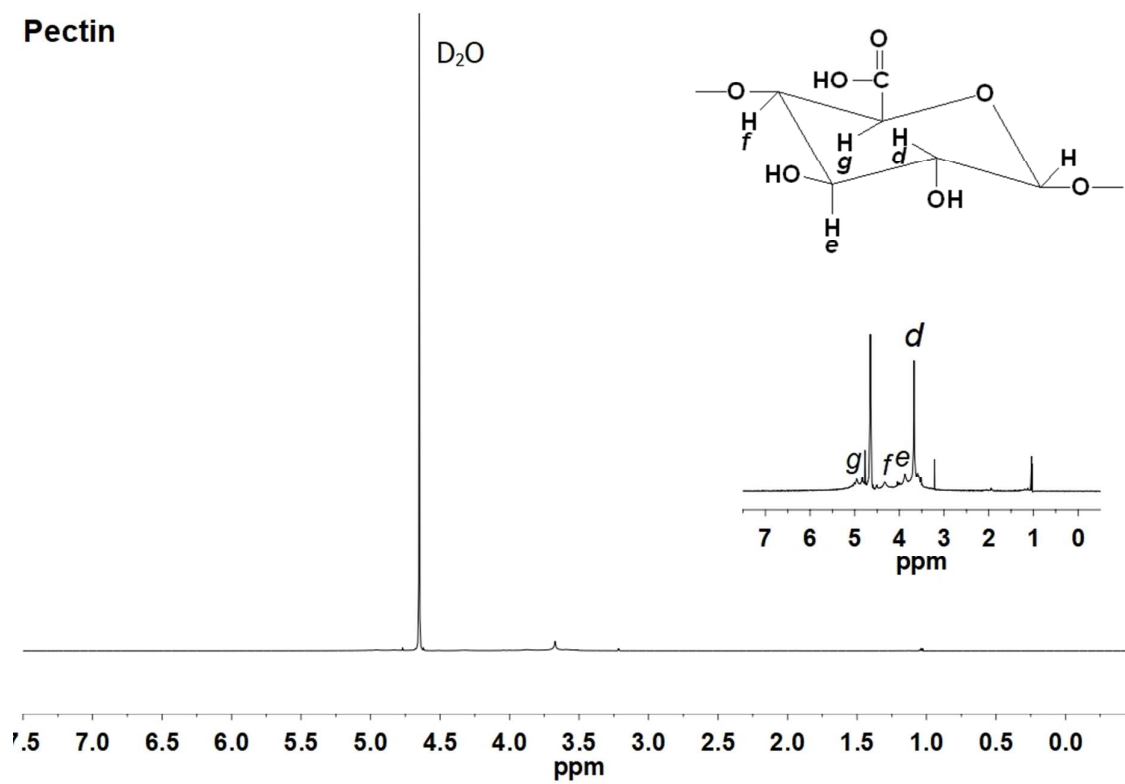


Figure S3.  $^1\text{H}$  NMR (500 MHz) spectrum of pectin.

**Table S1.** Thermal properties of the precursors (pectin and PHB) and of copolymer, pec-PHB.

<b>Sample</b>	<b>Thermal Decomposition Temperature, <math>T_d^A</math> (°C)</b>	<b>Residue<sup>B</sup> (%)</b>	<b>Glass Transition Temperature, <math>T_g^C</math> (°C)</b>	<b>Melting Temperature, <math>T_m^C</math> (°C)</b>	<b>Heat of Melting, <math>\Delta H_m^C</math> (J/g)</b>	<b>Degree of Crystallinity, <math>X_c^D</math> (%)</b>
<b>Pectin</b>	245.61	27.69	34.78	-	-	-
<b>PHB</b>	288.44	1.11	8.69	160.98	76.96	52.71
<b>Pec- PHB</b>	246.26	15.31	-15.41	-	-	-

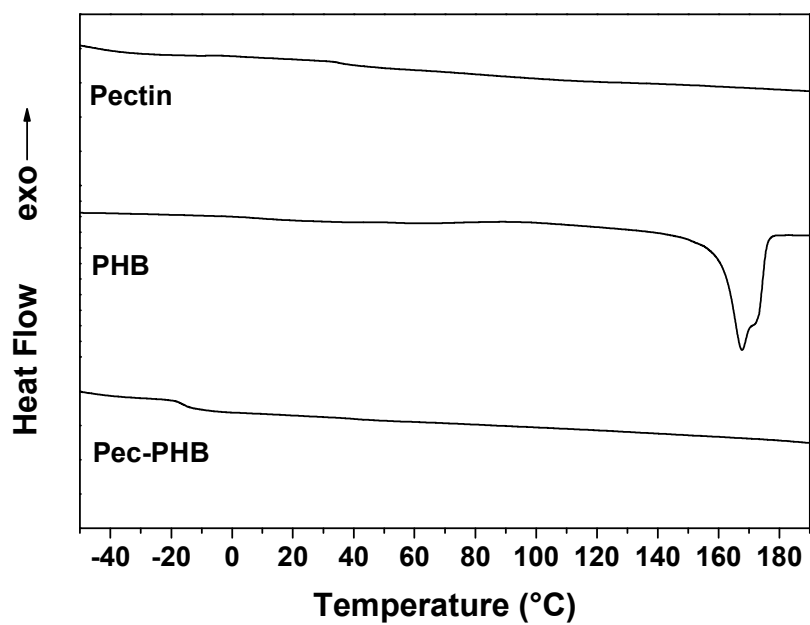
<sup>A</sup>  $T_d$  is defined as the temperature at which the mass of the sample has a 15% weight loss, determined from the peak of derivative weight curve by TGA. <sup>B</sup> Residue is defined as the mass percentage of the sample at 500 °C, determined from TGA. <sup>C</sup>  $T_g$ ,  $T_m$  and  $\Delta H_m$  were deduced from the second heating curve by DSC.  $T_m$  was taken as peak maxima.  $\Delta H_m$  was determined from the endothermic melting peak. <sup>D</sup>  $X_c$  was calculated using the following equation:

$$X_c = \frac{\Delta H_m}{\Delta H_m^O} \times 100\%$$

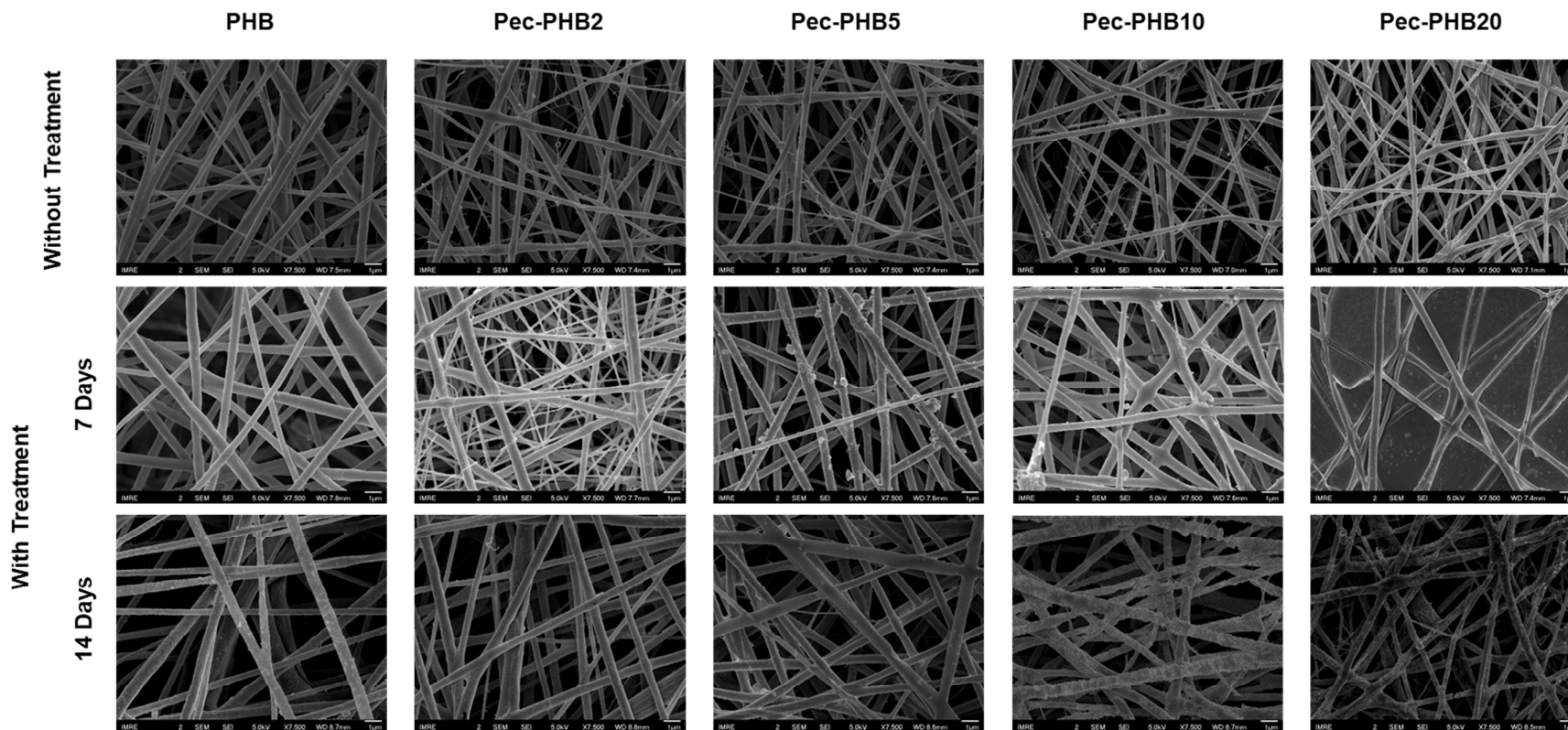
where  $\Delta H_m^0$  is a reference value which represents the heat of melting for 100% crystalline PHB, 146 J/g.<sup>1</sup>

### **Reference**

1. Barham, P. J.; Keller, A.; Otun, E. L.; Holmes, P. A., Crystallization and morphology of a bacterial thermoplastic: poly-3-hydroxybutyrate. *Journal of Materials Science* **1984**, *19* (9), 2781-2794.



**Figure S4.** DSC curves of the precursors (pectin and PHB) and of copolymer, pec-PHB.

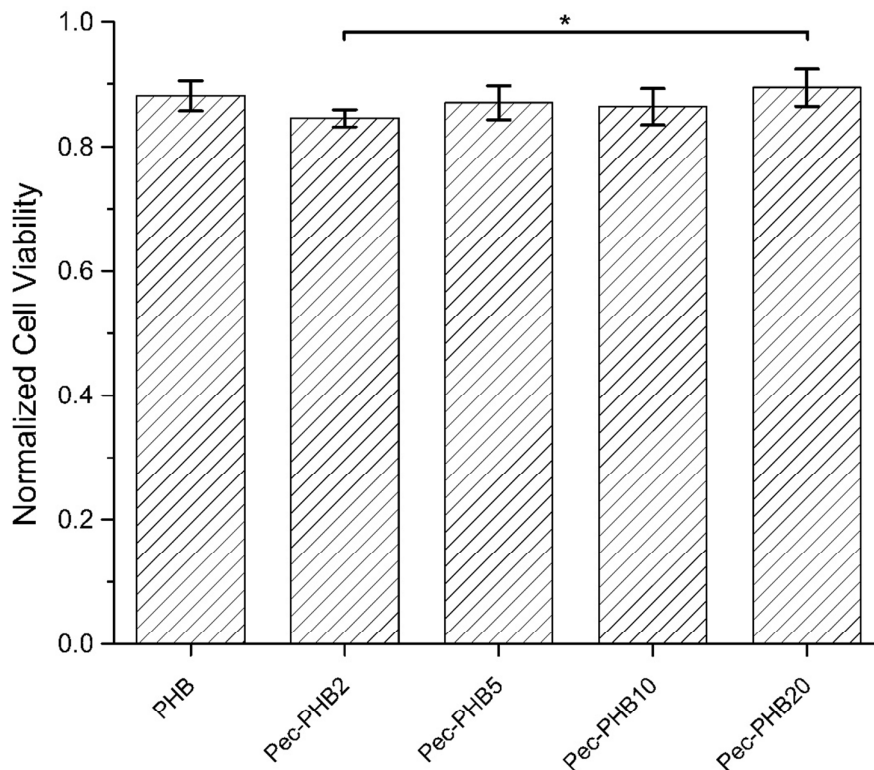


**Figure S5.** SEM micrographs (7500X) of electrospun fibres (PHB, pec-PHB2, pec-PHB5, pec-PHB10, pec-PHB20) without and with treatment (incubated in PBS solution (pH 7.4) at room temperature and lightly shaken at 150 RPM for 2 weeks) for 7 days and 14 days.

The different blends of our PHB/pec-PHB nanofibers were incubated in PBS solution (pH 7.4) at room temperature and lightly shaken at 150 RPM for 2 weeks with PHB nanofiber as the control. PBS solutions were changed every 2 days. SEM images of the nanofibers were acquired on



a weekly basis and revealed that the macrostructure of the fibre matrices remained intact in all cases. Incubating of the nanofibers in PBS solutions (pH 7.4 and pH 12.0) at 37 °C indicated complete solubilisation after approximately 2 months at the higher pH and 3 months at pH 7.4. Nanofibers with the higher pec-PHB content degraded faster under these conditions.



**Figure S6.** Cell viability of NIH/3T3 fibroblasts on PHB and pec-PHB nanofibers after 72 hrs. Readings are normalized to tissue culture plastic. (N = 6 | solid line:  $p < 0.05$ ).

Pec-PHB nanofibers were cultured with NIH/3T3 fibroblasts for 72 hrs and resazurin assay was performed to compare biocompatibility of the various nanofibers. Pec-PHB nanofibers present cell viabilities of 85% and above, comparable to that of pristine PHB. One-way ANOVA (Tukey post hoc test) at 5% level revealed that the means of pec-PHB2 and pec-PHB20 were significantly different, indicating higher pec-PHB content improved the cell viability. Overall, pec-PHB nanofibers were found to be non-cytotoxic and biocompatible.