SUPPLEMENTARY INFORMATION

An Environmentally Benign Approach to Achieving Vectorial Alignment and High Microporosity in Bacterial Cellulose/Chitosan Scaffolds

Guohui Li,^a Avinav G. Nandgaonkar,^{a,b} Youssef Habibi,^c Wendy E. Krause,^d Qufu Wei ^{a*} Lucian A. Lucia^{a,b,d*}

^aKey Laboratory of Eco-Textiles, Ministry of Education, Jiangnan University, 1800 Lihu Avenue, Wuxi, Jiangsu Province, Wuxi 214122, P.R. China

bKey Laboratory of Pulp and Paper Science & Technology, College of Light Chemical & Environmental Engineering, Qilu University of Technology, Jinan, P.R. China 250353
^cDepartment of Advanced Materials and Structures, Public Research Centre Henri Tudor, 5 rue
Bommel /ZAE Robert Steichen, 4940, Hautcharage, Luxembourg

^dFiber and Polymer Science Program, North Carolina State University, 2401 Research Dr., Campus Box 8301, Raleigh, NC 27695-8301

E-mail: lalucia@ncsu.edu, Telephone: +1 919.515.7707, Fax: +1 919.515.6302

Figure S1. and Figure S2.

BC and BC-Chitosan composites were subjected to a slow freezing process using a ultra-cold freezer (Sanyo, Japan); the samples were placed in a freezer before which ended up producing large random ice crystals; ¹ after which, all the samples were subjected to freeze drying shown in Figure S1.

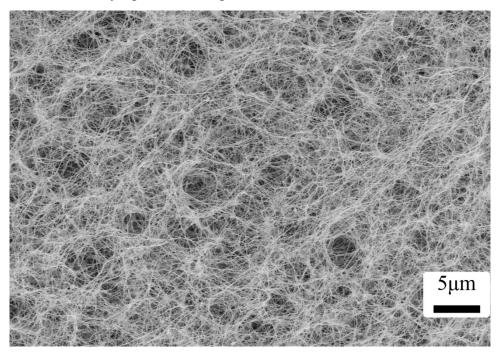


Figure S1. SEM microphotograph of BC produced by *Komagataeibacter xylinus* that were freeze dried by a slow freezing process. Note the presence of large pores and the absence of ostensible alignment.

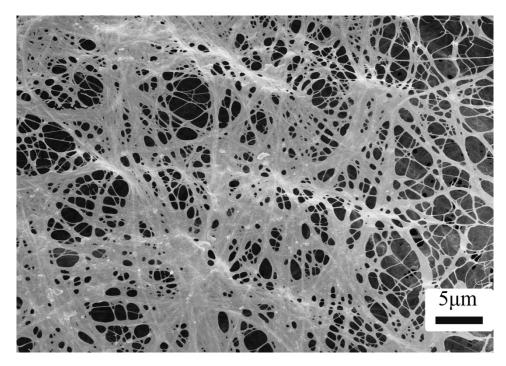


Figure S2. SEM microphotograph of BC-Chitosan composites that were freeze dried by a slow freezing process. Note the presence of large pores and the absence of ostensible alignment.

Figure S3. Determination of eutectic point using DSC

DSC (model Q200, TA Instruments, New Castle, Del.) was applied under nitrogen atmosphere at a flow rate of 20 mL min-1. Samples (\sim 13 mg) were equilibrated at -30 °C for 2 min. and heated to -20 °C at a heating rate of 10 °C min-1.²

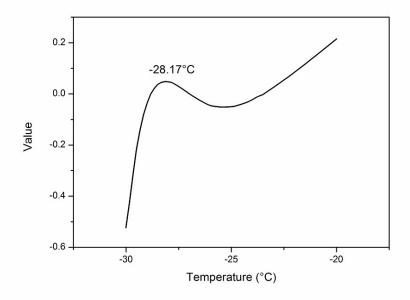


Figure S3. Determination of eutectic point by DSC.

Table S1. Selected physical properties of all samples.

Sample Name	Mean pore diameter (μm)	Porosit y (%)	Absorption properties (%)	Surface Area (m²/g)	Crystallinity Index (%)	Dry Compressiv e Modulus (MPa)	Wet Compressiv e Modulus (MPa)
BC	2 ± 1	75 ± 2	4671	201	89.45	9.51±0.23	37.32±1.07
BC-Ch-1%	4 ± 2	87 ± 1	1625	29.5	83.57	8.04±0.47	0.58±0.17
BC-Ch-1.5%	2.5 ± 1	80 ± 1	1532	22.06	83.94	9.13±0.40	0.82±0.15
BC-Ch-2%	1.4 ± 1	74 ± 2	1246	19.85	79.46	4.74±1.00	9.64±0.46

MTT test and Schwann cell culture

Per 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay (Fig. S4), the membrane extract demonstrated good biocompatibility as indicated by good cell viabilities compared to that of the control group during the different time.³

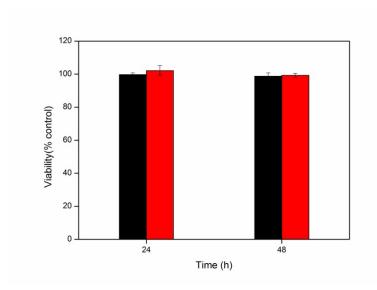


Fig. S4. The MTT test in different time (black represents blank control, red for membrane).

TBO staining was utilized to observe the Schwann cell behavior on the samples (Fig. S5), which showed the cells had spindle shape with projections from the first day to the third day of culture, and the cells were homogeneously distributed on the hydrogels surface, these results indicated that the scaffold is suitable for Schwann cells attachment and growth. ⁴

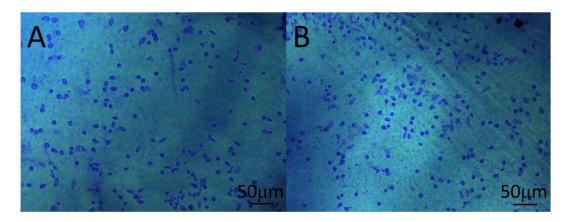


Fig. S5. Attachment and proliferation of Schwann cells on scaffolds (A 1d, B 3d).

References

- 1. Yu, S.; Zhang, Y.; Li, H.; Wang, Y.; Gong, C.; Liu, X.; Zheng, X.; Kopparapu, N. Effect of freeze-thawing treatment on the microstructure and thermal properties of non-waxy corn starch granule. Starch-Starke **2015**, *67* (11-12), 989-1001.
- 2. Ye, Y.; Cheng, M.; Xie, X.; Rick, J.; Huang, Y.; Chang, F.; Hwang, B. Alkali doped polyvinyl alcohol/graphene electrolyte for direct methanol alkaline fuel cells. J. Power Sources **2013**, *239*, 424-432.
- 3. Li, G.; Wang, Q.; Lv, P.; Ding, Z.; Huang, F.; Wei, Q.; Lucia, L. Bioremediation of Dyes Using Ultrafine Membrane Prepared from the Waste Culture of Ganoderma lucidum with in-situ Immobilization of Laccase. BioResources **2016**, *11(4)*, 9162-9174.
- 4. Li, G.; Kong, Y.; Zhao, Y.; Zhao, Y.; Zhang, L.; Yang, Y. Fabrication and characterization of polyacrylamide/silk fibroin hydrogels for peripheral nerve regeneration. J. Biomater. Sci. Polym. Ed. **2015**, *26(14)*, 899-916.