Polyhydroxyalkanoates (PHAs) and Bacterial Cellulose (BC), highly versatile natural polymers; their use in nerve tissue engineering

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Abstract

There is currently a high demand for best-fit materials for specific bulk and biomedical applications including nerve tissue engineering. In view of their sustainability and biodegradability, biobased green materials are more desirable for these purposes. Polyhydroxyalkanoates (PHAs) and Bacterial cellulose (BC) are among the best studied biomaterials in recent times. This study resulted in an average wet BC yield of 662 g/L and an yield of 40% dry cell weight (dcw) for the PHAs.

Introduction

Peripheral nerve injuries (PNIs) beyond **5-10 mm** length have poor **regenerative capacity.** It is estimated that an annual health care cost on nerve injury related cases is about USD 150 billion in the USA alone. Currently, autografts are the current 'gold standard' for PNI repair. This however leads to donor site morbidity among other deleterious drawbacks. Functional artificial nerve guide conduits (NGCs) (Figure 1) are more promising in addressing both short and critical gaps. PHAs and BC have demonstrated excellent properties including biocompatibility, bioresorption and suitable mechanical properties desirable for NGCs

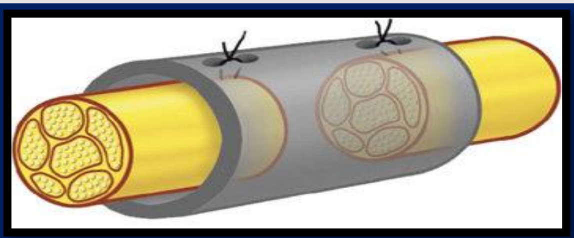
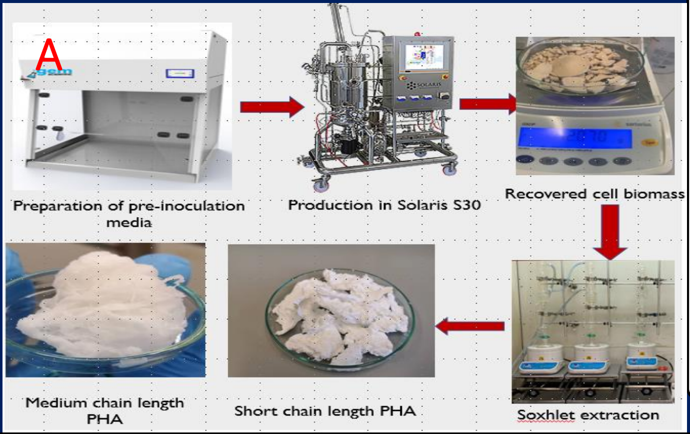


Fig. 1 Model NGC to be 3D printed with PHAs and BC. Adapted from Lizarraga *et al*. (2016)

Results

1. Production of PHAs and BC

A medium chain length (MCL) PHA, P(3HO-co-3HD and a short chain length PHA, P(3HB) were produced using *P. putida* K2440 and *B. subtilis* OK2 respectively, in a 30 L Solaris bioreactor, as illustrated in Figure 2(A). The MCL-PHA production resulted in a high yield of 40% dcw and productivity of 0.66 g/L/hr (about 15 times above literature values). Similarly, BC was cultivated under static conditions using *G. xylinus*. BC was produced with an yield of 662 g/L (Figure 2B)



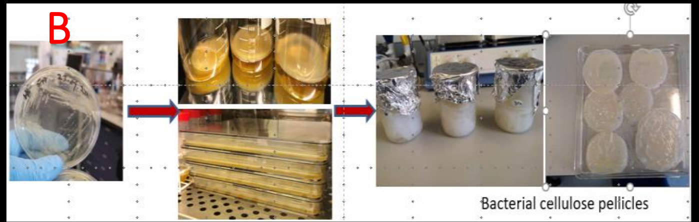


Fig. 2. **(A)** Production of PHAs using 30 L Solaris fermenter **(B)** Production of BC under static conditions

2. Scaffold Processing

Two dimensional films were prepared by solvent casting method using different blend proportions of PHAs for initial biocompatibility testing (Figure 3 D and E). Autodesk Fusion software was used to design various NGCs with different topographical cues as represented by Figure 3B and these structures were printed using the Cellink Bio X printer (Figure 3 A).

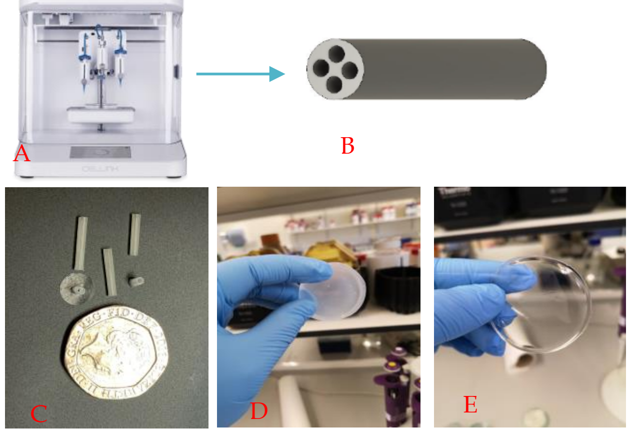


Fig. 3 A. 3D printer B. CAD design C. 3D printed PHA/PLA blend, (B) Solvent cast film of P(HB), a SCL-PHA and (C) Solvent cast film of P(3HO-co-3HD), an MCL-PHA

3. Biocompatibility Studies

The solvent cast films of PHAs were tested for their biocompatibility with NG108 neuronal cells using tissue culture plastic (TCP) as control. By day 6, the films of P(3HO-co-3HD) showed the highest cell proliferation.

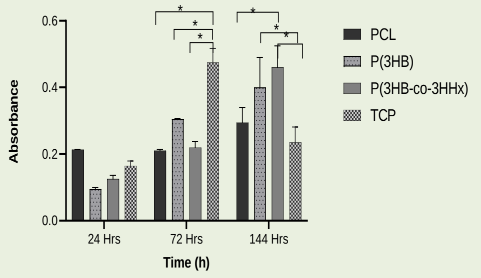


Fig. 4. Biocompatibility test of PHAs using NG108 neuronal cells with PCL and TCP as controls. N=3, n=3

Summary

PHAs and BC were successfully produced with over 15-fold increase in yield in both cases compared to those in published studies

PHA films supported neuronal cells with good biocompatibility

CAD designs were successfully test 3D printed at desired tube dimensions using PHA/PLA blends

References

Lizarraga -Valderrama, L. R. et al., (2016). Biomedical Applications of Polyhydroxy-alkanoates. Biomaterials from Nature for Advanced Devices and Therapies, https://doi.org/10.1002/9781119126218.ch20

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