A next generation bioinspired device for effective peripheral nerve regeneration

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Background

- Peripheral nerve injuries (PNIs) beyond **5-10 mm** length have poor **regenerative capacity**
- 200,000 and 300,000 cases require surgeries yearly in the USA and Europe respectively
- Annual health care cost on Nerve Injuries related cases is about USD 150 billion in the USA alone
- Many injuries require surgical nerve reconstruction
- Autografts are the current 'gold standard' for PNI repair
- This however leads to donor site morbidity.
- There is an urgent need for a better artificial nerve guide conduits that addresses the critical gap

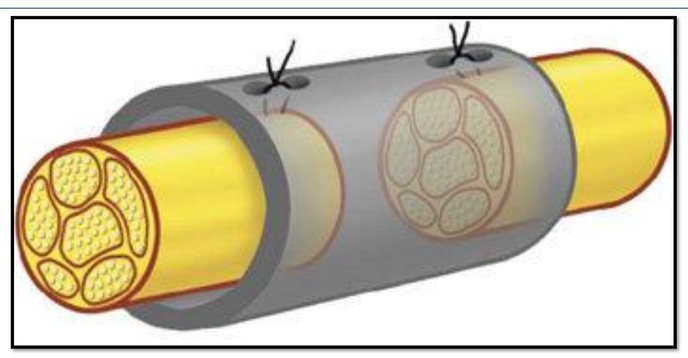


Fig. 1 Single lumen tube. Adapted from Lizarraga *et al*. (2016)

- Polyhydroxyalkanoates (PHAs) are ideal candidates for fabricating NGCs
- PHAs belong to family of natural polymers synthesized by bacteria.
- PHAs are biocompatible and biodegradable thermoplastic polymers
- PHAs are structurally diverse and with tuneable physical characteristics and easy processability.
- Similarly, Bacterial cellulose (BC) is a microbial synthesized hydrogel
- BC is highly porous, crystalline nanofibrillar structure with high water retention capacity
- BC is biocompatible and has excellent mechanical properties
- BC is Ideal for many biomedical applications

PREVIOUS WORK

- Shown that blends of PHAs; P(3HB)/P(3HO) (75:25) were superior to the widely commercialized PCL and other blending proportions of the PHAs in terms of biocompatibility with neuronal cells [2]
- Electrospun fibres of P(3HO)/P(3HB) (25:75) showed topographical migration in the direction of the fibres [1]
- Different PHA blend fibre groups supported growth and guided neuronal cells linearly towards the direction of the fibres and revealed a direct relationship between the fibre diameter and neuronal growth and differentiation [1]

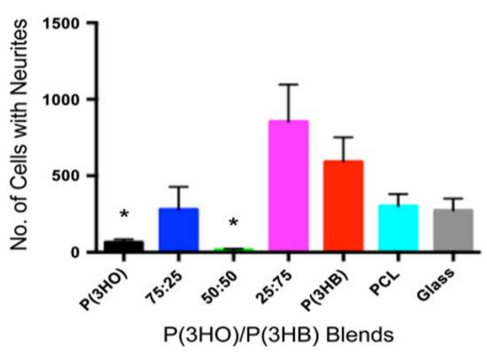


Fig. 2. NG108 proliferation on PHAs and PHA blends

AIMS

We propose to develop functionalized PHA and BC based advanced NGC to address longer critical gaps by increasing regeneration distance by:

- Producing the blended PHAs proportionally that will form nano/micro internal topography as well as BC
- Controlled release of active factors
- Processing of the NGC using the dip moulding, electrospinning and 3D printing techniques
- Evaluation of the final product using a dorsal root ganglion 3D *in vitro* chick model and mouse/rat *in vivo* models

METHODS

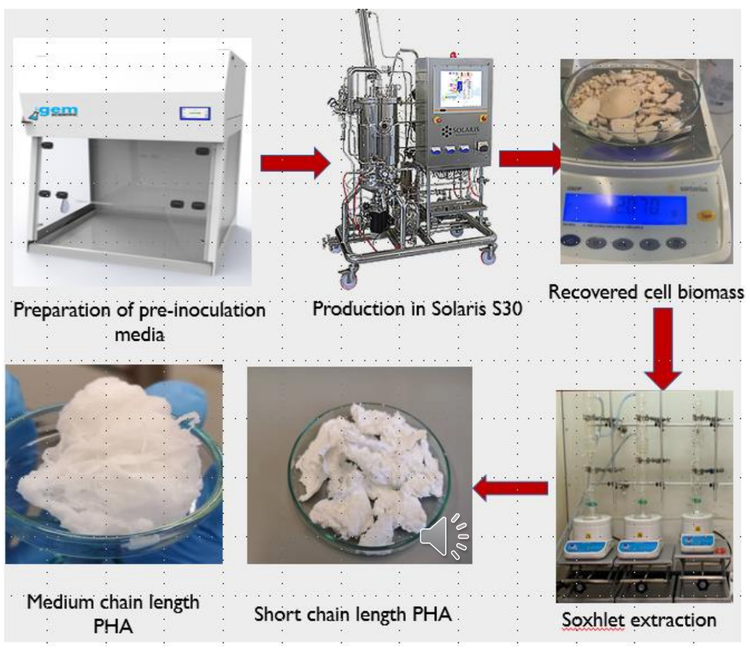


Fig. 3 Microbial production of PHAs using a 30 L Solaris bioreactor with a working volume of 20 L

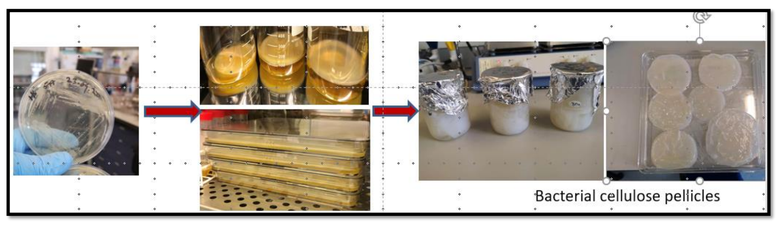


Fig. 4 Bacterial cellulose production using the static culture method.

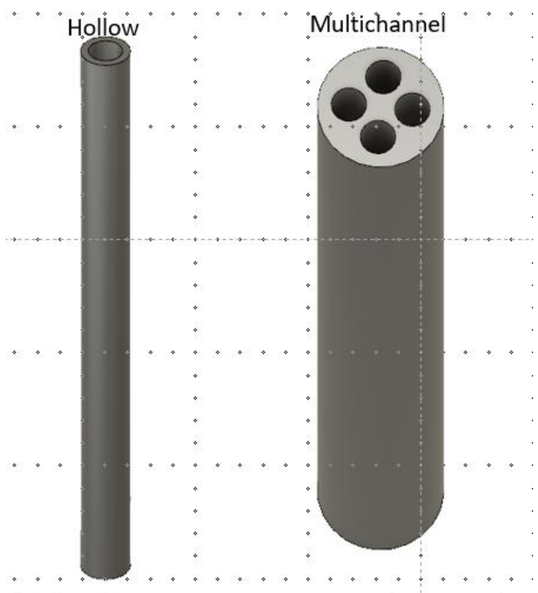
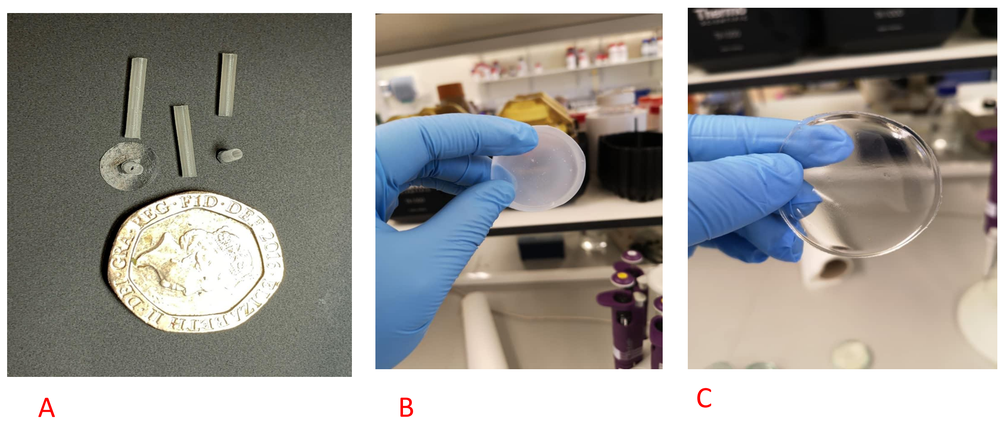


Fig. 5 CAD designs of nerve tubes with various topographical cues ranging from hollow to multichannels

RESULTS



A B C

Fig. 6. Processed PHAs. (A) 3D printed PHA/PLA blend, (B) Solvent cast film of P(HB), a SCL-PHA and (C) Solvent cast film of P(3HO-co-3HB), an MCL-PHA

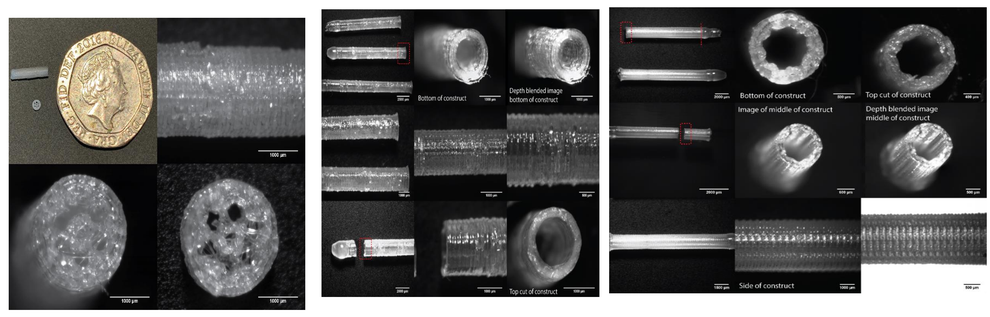


Fig. 7. SEM images of test printed PHA blends (a) Multichannel 3D printed PHA/ PLA bend b) Hollow 3D printed PHA/PLA blend and (c) Grooved 3D printed PHA/PLA blend

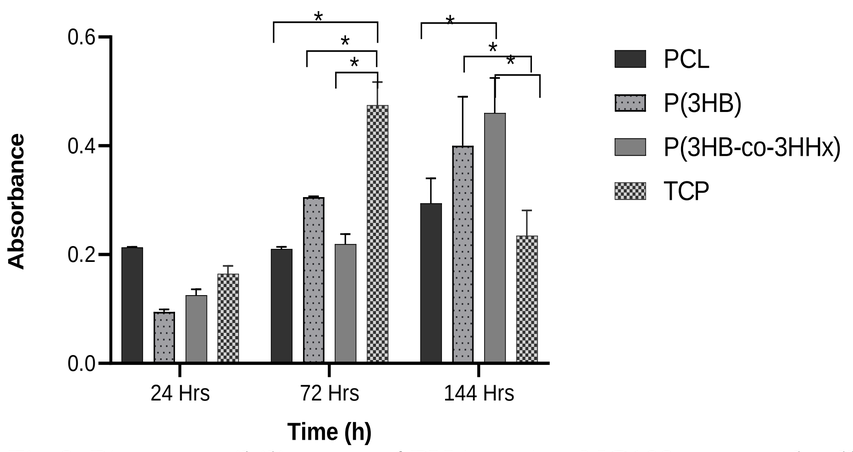


Fig 8. Biocompatibility test of PHAs using NG108 neuronal cells with PCL and Tissue Culture plastic as controls. N=3, n=3

Summary

- PHAs and BC were successfully produced
- PHA films supported neuronal cells with good biocompatibility
- CAD designs were successfully 3D printed at desired tube dimensions using PHA/PLA blends

Conclusion and Future work

- Overall, it has been confirmed that PHAs support the growth of NG108-15 neuronal cells.
- Initial biocompatibility of BC and preferred PHA blends will be assessed.
- The ability of the polymers to support the maturation and differentiation of NG108-15 neuronal cells will be assessed.
- Further work will be done using rat primary Schwann cells and dorsal root ganglion, as well as *in vivo* work using a Rat sciatic nerve injury model

References

1. Lorena R. Lizarraga-Valderrama *et al*., 2019, *Journal of Tissue Engineering and Regenerative Medicine*, Nov 8 https://doi.org/10.1002/term.2911

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