Incorporation of native-like extracellular matrix in nerve guide conduits for peripheral nerve repair

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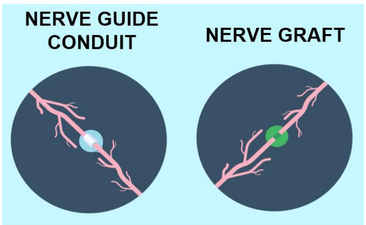
1. PROBLEM

- Peripheral nerve injuries can lead to **loss-of-function**, **long-term disability** and **neuropathic pain**.
- Nerve guide conduits (NGCs) can be used to repair and bridge nerve injuries, however, current NGCs do not provide adequate levels of regeneration, therefore novel NGCs are in high demand.
- Studies suggest the **incorporation of extracellular matrix components** can improve nerve regeneration (1).

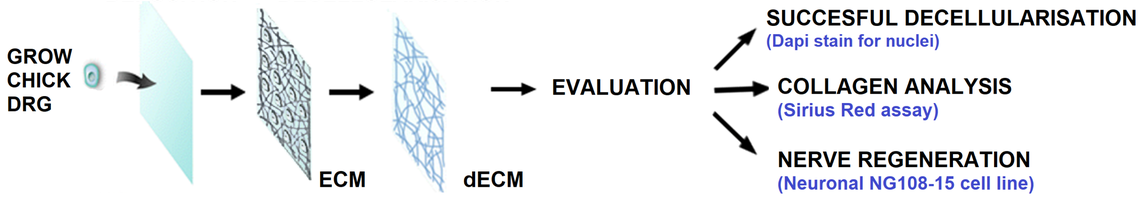


2. HYPOTHESIS AND AIMS

Dorsal root ganglia, a source of primary nerves, can be used to deposit a native neuronal ECM due to the presence of neuronal, glial and non-glial cells within the ganglia. The cellular components can be removed by decellularisation, resulting in a naturally-obtained decellularised ECM (dECM). The dECM can serve as a novel substrate for nerve regeneration.

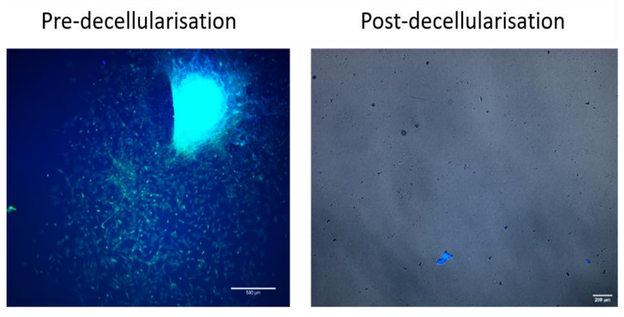
i. **Deposition of chick DRG-derived ECM and effective decellularisation, maintaining ECM integrity**
ii. **Evaluation of dECM *in vitro* with neuronal NG108-15 cells**

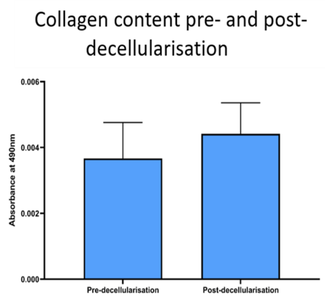
3. METHODS



4. RESULTS

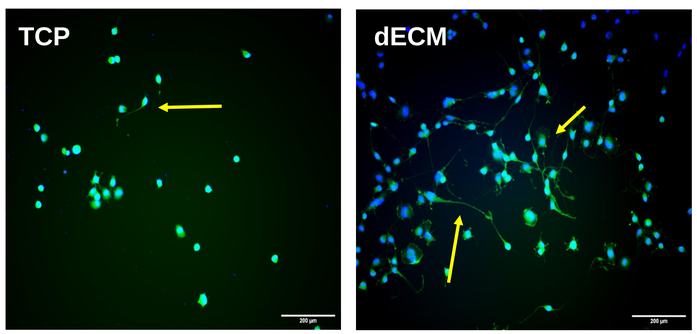
i) DRG-ECM was successfully decellularised maintaining collagen content





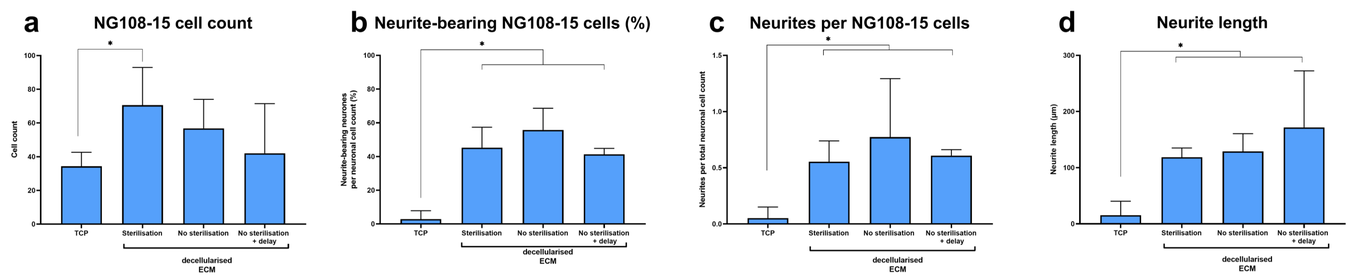
Chick DRGs were used to deposit extracellular matrix on TCP surfaces (pre-decellularisation). After decellularisation (post-decellularisation), no DAPI- or Phalloidin-stained cells could be seen, demonstrating successful decellularisation. The collagen content measured in the DRG-derived ECM was not significantly affected during decellularisation (Graph). *Blue: DAPI stain for nuclei, Green: Phalloidin stain for F-actin filaments. Scale bar 500µm and 200µm.*

ii) Neuronal attachment and neurite outgrowth was improved on decellularised ECM surfaces compared to TCP



NG108-15 neuronal cells were seeded and cultured for 1 week on tissue culture plastic (TCP) and on the decellularised ECM surfaces (dECM).

More neuronal NG108-15 cells were present on the dECM compared to TCP. As well, more neurites were extended on dECM surface, with longer neurites compared to TCP. *Arrows show neurites. Blue: DAPI stain for nuclei, Green: Phalloidin stain for F-actin filaments. Scale bar 200µm*



a) The number of neuronal NG108-15 cells was higher on dECM surfaces compared to TCP, with one significant increase on the sterilised dECM.
b) The percentage of neurite-bearing NG108-15 cells was significantly increased on all dECM surfaces compared to TCP.
c) More neurites (normalized to cell count) were expressed on dECM surfaces compared to TCP.
d) Neurite length was significantly increased on dECM surfaces compared to TCP.

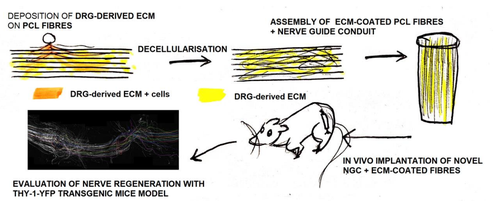
5. CONCLUSIONS

The decellularised chick-derived ECM can serve as a novel substrate for nerve regeneration, as

- Collagen content in the ECM is maintained during the decellularisation protocol
- Neuronal NG108-15 cells show **increased attachment, neurite extension and neurite length** on dECM surfaces compared to TCP.

6. FUTURE WORK

- Deposition of ECM on electrospun fibres followed by decellularisation
- *Ex vivo* assessment of dECM-coated fibres with 3D model using a mouse-derived DRG.
- *In vivo* implantation of the novel NGC and assessment of axonal regeneration using a Thy-1-YFP transgenic mouse model (right) (2)



References. (1) Bell, J. H., & Haycock, J. W. (2011). Tissue Engineering Part B: Reviews, 18(2), 116-128. (2)Singh, D., (2018). Acta biomaterialia, 78,48-63.