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Decellularisation of Human Femoral Nerves in a Closed System: Towards Introducing a New Nerve Allograft in Healthcare in the UK

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Introduction

In the UK, there is currently a high medical need for human nerve allografts to treat peripheral nerve regeneration occurring after traumatic injuries. The implantation of an autologous nerve graft, or an imported allograft from the US, both present limitations and a high NHS cost. We aimed to develop a new nerve allograft by decellularising human femoral nerves from deceased donors in the UK, while validating a new closed-system for processing tissue to meet GMP requirements in national tissue banking.

Methods

Twelve femoral bundles were retrieved from 6 independent donors within 48 hrs of death (age range: 20-66 years old; 1:1 male to female), and 25-30 cm long native nerves were dissected out. One nerve segment (min 6 – max 25 cm) from each donor was decellularised by a series of hypotonic, mild detergent, nuclease and hypertonic steps over a total of 5 days at 37-42°C in a closed system bag (CryoMACS). Histological analyses were performed on native versus decellularised nerve biopsies and included: Van Gieson, Sirius Red, DAPI, Fluoromyelin® and immunohistochemical staining. The absolute amount of residual double stranded DNA was measured by a PicoGreen assay on dried tissue. Biomechanical testing was performed by using a uniaxial pull-to-break assay on a Lloyds universal tester (100N load cell).

Results & Discussion

Human femoral nerves were successfully decellularised, with minimal residual DNA (mean ± SD: 2.44 ng/mg ± 1.62; $p < 0.001$) below the acceptable limit of 50 ng/mg of dry tissue. Histology investigation revealed that nerve fascicle architecture has been preserved during decellularisation. Biomechanical properties of the nerve were not significantly affected (mean load at failure ± SD: 9.32N ± 8.7 vs 12.2N ± 7 in native vs decellularised nerves; $p>0.05$).

Conclusion

We successfully decellularised human femoral nerves from cadaveric donors in a sterile closed-system. Future work will include cytotoxicity tests *in vitro* and implantation into an animal model of peripheral nerve injury to prove safety and efficacy of the new graft. This study will ultimately lead onto a clinical evaluation of decellularised human nerves in the UK to repair peripheral nerve injuries.

Funding and Ethics

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