

In vitro Characterization Of Different Decellularization Protocols for Peripheral Nerve Grafts Preparation.

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Introduction:

In critical nerve gaps, where tensionless repair is unapplicable, a nerve graft or conduit are needed to connect the two nerve stumps. Decellularized nerve allografts are a promising tissue engineering strategy. Their superiority over nerve conduits is owed to the availability of natural well-conserved extracellular matrix (ECM) components that has proven to play an important role in supporting axonal guiding and peripheral nerve regeneration.

The known decellularization techniques nowadays are time and effort consuming. In the present work performed on rat sciatic nerves, we investigated a novel nerve decellularization protocol able to combine an effective decellularization in short time with a good ECM preservation.

Methods:

Two different decellularization protocols were tested. The first protocol - proven to be efficient for decellularizing tendons (DN-P1), using 1% tri(n-butyl) phosphate (TBP), 3% peracetic acid (PAA) (Lovati et al., 2016) - was compared with a decellularization protocol specifically developed for nerves (DN-P2), using 125mM SB-10, 0.2% TritonX-100, 0.25% SDS, and sonification cycles (Boriani et al.; 2017). The outcomes of both decellularization protocols were assessed by a series of in vitro evaluations, including qualitative and quantitative histological and immunohistochemical analyses, DNA quantification, SEM and TEM ultrastructural analyses, mechanical testing, and viability assay.

Results:

Both decellularization protocols had led to an overall well-preserved nerve structure; DNA quantification showed that DNA content was significantly decreased, but not completely removed.

Both protocols had less cellular component, but complete removal was not achieved; an adequate amount of ECM component is still conserved in both protocols. DN-P1 has better biomechanical properties, superior biocompatibility and ultrastructural properties compared to DN-P2.

Conclusions:

DN-P1 greatly demonstrated superior results compared to DN-P2 in terms of ultrastructural analysis and biocompatibility. Decellularized nerve allografts prepared following DN-P1 protocol are promising for long gap repair in vivo.

Special Interest Group:

Neuropathic Pain Consortium (NPC)

Biology:

Axon

Clinical:

N/A

Techniques:

Imaging

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No

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Field

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Prizes

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