Decellularization of peripheral nerve tissue with preservation of extracellular matrix using non-thermal irreversible electroporation

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Introduction:

Non-thermal irreversible electroporation (NTIRE) is a new minimally invasive surgical technique that was originally conceived from theoretical considerations with the capability of selectively targeting cell membranes to treat biological tissues. Short, microsecond electrical pulses are applied to the tissue, selectively targeting the cell membrane, causing pores to form **:** within the membrane and leading to cell death.

Material and Methods:

Irreversible electroporation (IRE) of femoral neurovascular bundles was performed. Also, electrophysiological, histological, and functional studies were performed. A sequence of pulses was applied directly to peripheral nerves.

Results and discussion:

After IRE, peripheral nerve function was damaged before four weeks, but then gradually returned to normal. Perineural inflammatory cell infiltration was most severe three days after IRE (80–85%), and was normalized after eight weeks. Surrounding tissue injury was prominent at three days and one week after IRE (80–90%), and then gradually recovered. However, peripheral nerve fibers were markedly damaged at one and two weeks (80–100%). Nerve fibers then recovered and were normalized at eight weeks.

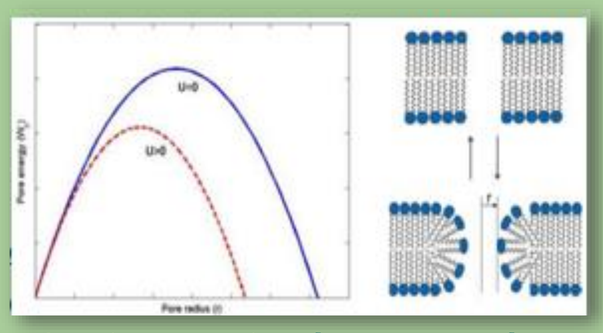
Conclusion:

Nerve tissue injury with transient functional impairment can occur after IRE. However, endoneurial and epineurial extracellular matrix were preserved with Schwann cell regeneration, which could lead to regeneration of nerve tissues within eight weeks. Therefore, we focused on the in vivo application of irreversible electroporation for biomedical purposes which is tissue decellularization.

Keywords:

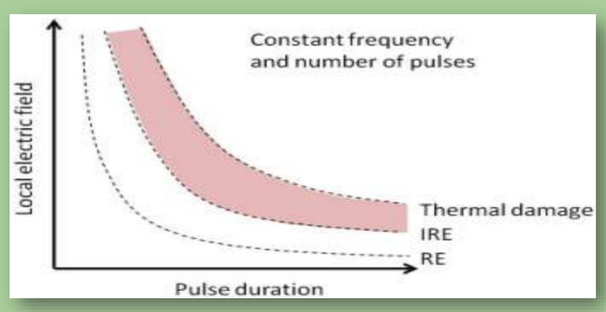
Electroporation, Tissue decellularization, Non-thermal irreversible electroporation, Peripheral nerve tissue electroporation

Energy barrier of pore formation in presence and absence of external electrical field in electroporation



- An increase in the transmembrane potential results in a decrease in the energy barrier for hydrophilic pore formation. Here, this is illustrated for the case when there is no applied transmembrane potential (U = 0) and for an elevated transmembrane potential case (U > 0).In addition, it is shown converting hydrophobic pores to hydrophilic ones.

Effect of electric field and pulse duration on cell response during electroporation.

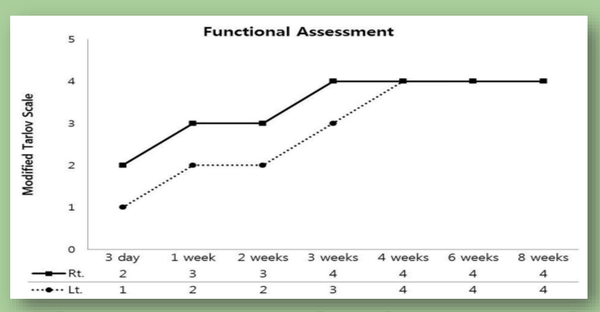


- Effect of local electric field can be seen in 4 different ways:

1. No effect: weak electric field
2. Reversible electroporation
3. Irreversible electroporation
4. 4thermal damage

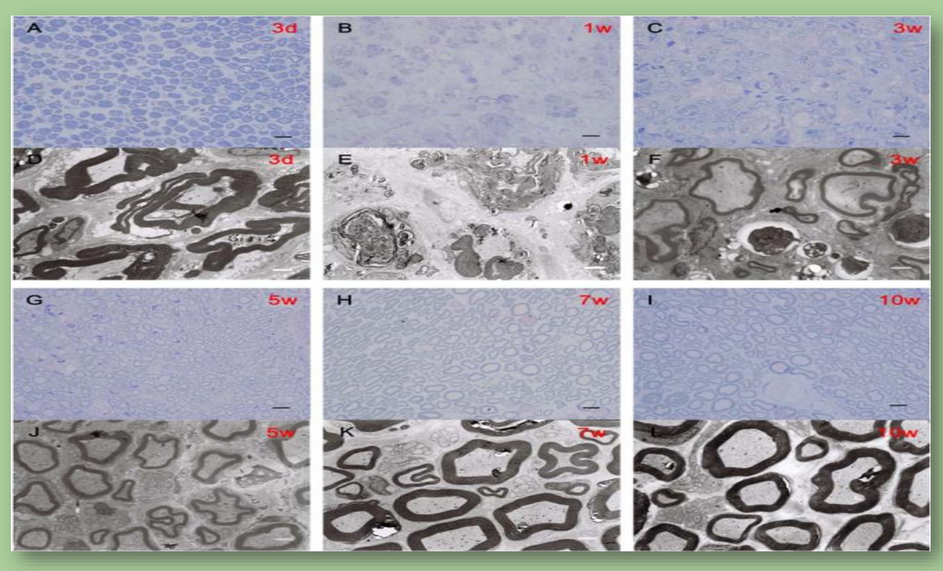
All above-mentioned processes depends on magnitude of the electric field.

Functional evaluation of rabbit legs after IRE



- The nerve tissue has been treated after 8 weeks.(right leg: 90 pulses, left leg: 180 pulses, and both 2000 V/cm with 90µ pulse duration)

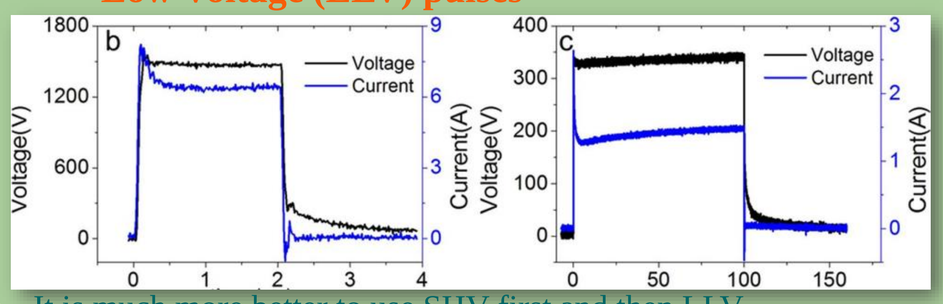
Remyelination of the nerve after IRE



- (a–c, g–i) Toluidine blue staining. Light micrographs of transverse semi-thin sections at the injury sites of IRE at 3 days, 1 week, 3, 5, 7 and 10 weeks after injury. (d–f, j–l) Transmission electron micrographs(TEMs). Ultra-thin sections at the injury sites of IRE at 3 days, 1 week, 3, 5, 7 and 10 weeks after injury were observed under TEM.

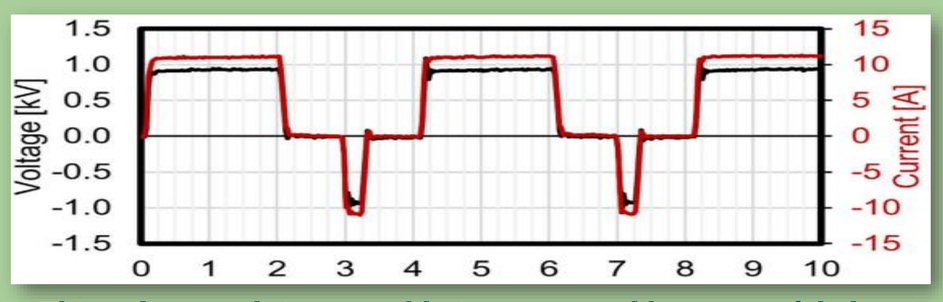
Two new methods to get better results in IRE

1. **Using combination of Short High Voltage (SHV) and Long Low Voltage (LLV) pulses**



- It is much more better to use SHV first and then LLV

2. **Using asymmetric polar pulses**



- This pulses result in more ablation zones and less powerful electric field is needed

Key points:

Overall, non-thermal irreversible electroporation can be used for tissue decellularization to preserve the extracellular matrix for growing new cells. As a new method, this preserved natural extracellular matrix would be used in some special situation which the peripheral nerve of a person has been damaged.
However, this method has been used for cancer treatment previously.

References:

1. Kwon JH, Kim MD, Kim SH, Lee EW, Kahlid SA. Effects of irreversible electroporation on femoral nerves in a rabbit model. Minimally Invasive Therapy & Allied Technologies. 2022 Feb 1;31(2):306-12 Kwon JH, Kim MD, Kim SH, Lee EW, Kahlid SA. Effects of irreversible electroporation on femoral nerves in a rabbit model. Minimally Invasive Therapy & Allied Technologies. 2022 Feb 1;31(2):306-12
2. Yao C, Lv Y, Zhao Y, Dong S, Liu H, Ma J. Synergistic combinations of short high-voltage pulses and long low-voltage pulses enhance irreversible electroporation efficacy. Scientific reports. 2017 Nov 9;7(1):1-6.
3. Li W, Fan Q, Ji Z, Qiu X, Li Z. The effects of irreversible electroporation (IRE) on nerves. PloS one. 2011 Apr 14;6(4):e18831.
4. Asymmetric Waveforms Decrease Lethal Thresholds in High Frequency Irreversible Electroporation Therapies
5. Phillips MA. The Molecular Selectivity of Non-Thermal Irreversible Electroporation and Tissue Regeneration In Vivo. University of California, Berkeley; 2012.