Title: **Preparation and performance evaluation of electrospinning bilayer nerve conduit based nerve extracellular matrix and PLA-PCL**

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Abstract:
Extracellular matrix (ECM) is derived from porcine nerves, which had a good performance as nerve repair biomaterial. However, decellularized porcine nerves (DPN) have limitations including lacking sufficient mechanical support, and the defined size of DPN difficult to fit the nerve defect. In this study, a novel electrospinning bilayer structure nerve conduit（BNC）, which based on poly (L-lactic acid-co-ε-caprolactone) (PLA-PCL) and ECM, was fabricated for nerve regeneration. Electrospinning ECM was considered as the inner layer of BNC, and PLA-PCL was electrospun into an outer layer. The results showed that BNC retained some neural extracellular matrix components and bioactive molecules, including collagen I, collagen IV, laminin, fibronectin, glycosaminoglycan, NGF, and BDGF, demonstrated by immunehisto-chemistry, Western blot, and enzyme-linked immunosorbent assay. Scanning electron microscopy images showed that an inner layer composed of ECM by electrospinning had a network of nanofibers. Biomechanical analysis proved that electrospun PLA-PCL as an outer layer improved mechanical properties of BNC. In vitro, the biosecurity of BNC was evaluated by cytotoxicity, subcutaneous implantation test and cell affinity analyses. In vivo, BNC was implanted in a rat sciatic nerve mode of 10 mm long-distance defects for up to 12 weeks, in comparison with PLA-PCL conduit, ENC and autograft. The functional nerve recovery was assessed by functional analysis, electrophysiological analysis, and immunohistochemical analysis. These findings indicated that BNC not only retains the advantages of ECM, but also with the appropriate mechanical strength, which would be a great potential clinical nerve repair biomaterial for peripheral nerve injuries.