New Flexible Elastomer Nerve Conduit For Peripheral Nerve Injury Repair

WM Choy[1], RT Tran[2], KWK Yeung[1], J Yang[2], WY Ip[1]
Department of Orthopaedics and Traumatology, Queen Mary Hospital, The University of Hong Kong, Hong Kong SAR[1]
Department of Bioengineering, The University of Texas at Arlington, Arlington, TX 76019, USA[2]

Introduction: Peripheral nerve injuries are common clinical problems, and are associated with difficulties in self-regeneration. End-to-end suturing and autograft bridging are current treatments plagued with several limitations such as severe donor site morbidity, the need for multiple surgeries, stimulation of intranurel fibrosis, and limited supplies. These problems have motivated the development of artificial nerve guidance conduits (NGC). Although many NGCs made of different materials have been commercialized, their therapeutic benefits remain unsatisfactory. Our newly developed biodegradable crosslinked urethane-doped polyester elastomer (CUPE) maybe brings a breakthrough to the nerve regeneration field. CUPE's compliant, tunable, soft, yet strong mechanical properties provide an optimal material for the evaluation of ideal NGCs. CUPE has demonstrated great potential in soft tissue regeneration. However, its benefits over the commonly used materials such as PCL and PLLA for nerve regeneration have not been tested. In this study, we evaluated the cytocompatibility of porous and non-porous CUPE, PCL and PLA with Schwann cell culture.

Methods: Briefly, a purified poly(1,8-octanediol-co-citric) (POC) pre-polymer was prepared by a chemical reaction of citric acid and 1,8-octanediol in a 1:1.1 monomer ratio. The dissolved pre-polymer was reacted with 1,6-hexamethyl diisocyanate (HDI) in a feeding ratio of 1:1.2 such that the pre-CUPE was fabricated. CUPE films were fabricated by drying the pre-CUPE in a Teflon mold while the porous CUPE was synthesized by mixing pre-CUPE with pure sodium chloride 50-106 µm in (1:9) polymer to salt ratio by weight following by solvent evaporation and lyophilization. These CUPEs were then crosslinked in an oven maintained at 80°C. The poly-L-lactic acid (PLLA) and poly-ε-caprolactone (PCL) granules were melted and shaped into 5mm diameter disc. All the samples were sterilized by ethylene oxide (EtO) and then immersed into various solutions (different pH of ddH_2O, medium with 10% fetal bovine serum (FBS) and FBS) following by normal cell culture. Rat Schwann cells (SCs) (line RT4, CRL-2768) purchased from ATCC (Tin Hang Technology, HK) were cultured on the samples for 1, 4 and 7 days to determine cell proliferation and morphology. The MTT-proliferation assay was employed to quantify cell viability. In addition, the samples were fixed by standard fixation procedure and the cell morphology was studied by Scanning Electronic Microscopy (SEM).

Results: The result in Figure 1 demonstrates that the influence of pH value of immersion solution was not governing the result, but the FBS dramatically enhanced the cell activity. Figure 2 shows the SC viability as determined by MTT assay. The results indicated that PLA supported the best SC growth while the worst on PCL. The porous CUPE scaffolds displayed improved cell proliferation when compared to CUPE films. In Figure 3, the cell morphology from the PLA was well spread while fewer cells were observed from the PCL were observed. In addition, cell expressed pseudopodia into the pores was observed in the porous CUPE.

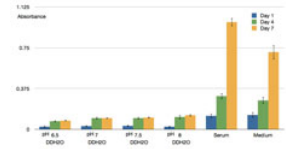

Fig.1 Absorbance of various pH and medium treated CUPE over time from the reading at 570nm and wavelength using the MTT assay. The reference wavelength of 640nm was used.

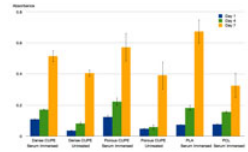

Fig.2 Absorbance of dense and porous CUPE, PCL and PLA over time from the reading at 570nm and wavelength using the MTT assay. The reference wavelength of 640 nm was used.

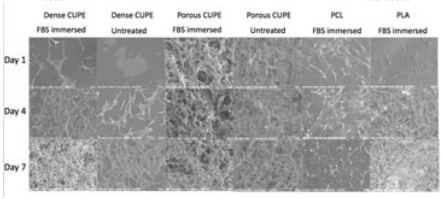

Fig.3 Cell morphology of dense and porous CUPE, PCL and PLA over time.

Discussion: FBS has a variety of proteins which adhered on the samples would help the cell adhesion and also its proliferation. We could postulate that the pre-adhered proteins provided extra nutrients to the later seeded cells. Therefore, a better cell activity was observed on the FBS immersed samples.

PLA displayed a higher potential for the use of NGC due to the higher cell proliferation and characteristic of Schwann cell morphology when compared to PCL and CUPE. However, PLA is too rigid to be implanted as a NGC and its mechanical property limits its application. Although flexible in nature, the commercially available PCL indicates its weakness with regard to cell compatibility. The performance of CUPE is not inferior to PLA and superior to PCL in term of cell proliferation and morphology. Nevertheless, its excellent flexibility is superior among the samples. We can summarize here that the CUPE could be a potential material of NGC.

The introduction of pores into CUPE provides a larger surface area for cell attachment and migration. Concerning the axonal regeneration mechanism, the migration of SC would favor the functional recovery due to the better development of bands of bünger. This experiment gives an outline of further development of CUPE-NGC by introducing micro-architecture so as to mimic the natural nerve structure. The micro-architecture would provide an alignment pathway for the axonal regrowth so as to achieve a better innervation. Further studies are needed to investigate the gene and protein expression of SC on the CUPE such that a more precise and quantified analysis on the suitability of CUPE as a NGC can be performed.

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