A Novel Flexible Nerve Guidance Conduit with micro-channels in Animal Models

Introduction:
Historically, there has not yet been a perfect artificial nerve guidance conduit (NGC) fabricated. The commercial available NGCs have their limitations due to material and structural deficiencies, and the critical length of functional repair is limited to three centimeters. For the defects under 3cm long, the nerve autograft is considered to be the golden choice of treatment however, there are a number of limitations including that of donor site morbidity, limited available supply and creation of second wounds on the patients. These all initiated the development of artificial NGCs, which are the alternative treatment of peripheral nerve system injury. The difficulties in achieving functional recovery from the use of commercial available NGCs are attributed to be the design of them. Previously, we have reported that the new flexible NGC made of our novel biomaterial, crosslinked urethane elastomer (CUPE) has excellent biocompatibility in in-vitro and in-vivo. In this study, we aim to investigate the axonal recovery of rats implanted with micro-architectural design of CUPE compared to that of nerve autograft and poly caprolactone tube.

Methods:
The poly (1,8-octanediol-co-citric) acid (POC) was prepared by mixing citric acid and 1,8-octanediol in a monomer ratio of 1:1.1. Afterwards, the pre-polymer was dissolved and reacted with 1,6-hexamethyldiisocyanate (HDI) at a feeding ratio of 1:1.2. The pre-CUPE was mixed with sieved sodium chloride (ranging between 50-106um). The mixed slurry was then put on a bundle of titanium rods (0.5mm diameter) and followed a post-polymerization at 80°C. Another slurry was rubbed as a sleeve and encapsulated the titanium rods with polymerized CUPE. Afterward, another 80°C post-polymerization was taken. A leaching process was carried in deionized water. Finally, the titanium rods were taken and the CUPE with porous sleeve (CUPE-S) were prepared. In this study, three controls: (a) porous CUPE tube (CUPE-T), a nerve autograft and a poly-caprolactone tube (PCL) were also prepared. Before the operation, all the samples were sterilized by ethylene oxide (ETO) and immersed into fetal serum for 4 days.

Four 3-month old Lewis rats were used in this study. A 1cm section of their right sciatic nerve was removed and replaced by the samples described previously. After eight weeks, the nerve grafts were removed, histological processed and sectioned and then evaluated by H&E and Toluidine blue staining. All the grafts were divided into 5 segments (a) proximal nerve, (b) proximal conduit, (c) central conduit, (d) distal conduit and (e) distal nerve. The myelinated fiber density, population and diameter were measured manually in six random areas (0.0111mm²) per section.

Results:
Figure 1 illustrates the intact samples from the proximal to the distal stump.

Figure 2 revealed the microscopic images of the central conduits in all groups. The PCL tube showed the poorest re-innervation of nerves.

Discussion and Conclusion
This study evaluated the performance of CUPE NGC with a designed micro-architecture in the axonal recovery of peripheral nerve system injury. Ideally, the micro-architecture is used to mimic the natural nerve structure so as to promote both axonal and functional recovery of peripheral nerve system injury. In the rat models, all the animals did not show any inflammation throughout the eight weeks investigation, which indicates that CUPE is an excellent and biocompatible material with potential uses in tissue engineering. During the operation, the soft and flexible CUPE is easily handled. The external porous sleeve not only provided an area for encapsulating the injured stump as shown in figure 3, but also provide further mechanical support to the NGC. In addition, the success in re-innervation of nerve was observed in all groups. By comparing the nerve population in the central conduit, it seemed that the results from the CUPE-S achieved the least difference from that of the nerve autograft.

From the microscopic images, the collapse of micro-architecture was observed in CUPE-S. It provides information on the needs of tackling the collapse for further development. Thus, further studies are required to enhance the toughness of micro-architecture. Besides, the performance of CUPE-S shows equally as effectively as the nerve autograft does. This pilot study successfully proved that the introduction of micro-architecture in the CUPE-NGC can enhance the nerve regeneration in terms of the fiber density and population. In the future, we need to improve the sustainability of micro-architecture so as to prevent the collapse of it during the implantation. Also a precise functional recovery study should be done so as to prove the efficiency of micro-architectural CUPE-NGC.

Acknowledgement
This project was financially supported by HKU Seed-funding for applied research #21000-301-01.

Table 1 shows that the myelinated fiber population and density of the CUPE-S is similar to that of nerve autograft at the section of central conduit.

Table 1 Summary of fiber population, fiber density and fiber diameter in the central conduit of (a) CUPE-S, (b) CUPE-T, (c) nerve autograft and (d) PCL tube. (Red in colour: the p-value comparing with results in CUPE-S is <0.05)

<table>
<thead>
<tr>
<th>Groups</th>
<th>Fiber Population (Fiber/mm²)</th>
<th>Fiber density (um)</th>
<th>Fiber diameter (um)</th>
</tr>
</thead>
<tbody>
<tr>
<td>(a) CUPE-S</td>
<td>143 ± 27</td>
<td>12,914 ± 2,454</td>
<td>0.774 ± 0.17</td>
</tr>
<tr>
<td>(b) CUPE-T</td>
<td>85 ± 21</td>
<td>7,688 ± 1,859</td>
<td>0.740 ± 0.20</td>
</tr>
<tr>
<td>(c) Nerve Autograft</td>
<td>120 ± 26</td>
<td>10,872 ± 2,318</td>
<td>0.8723 ± 0.16</td>
</tr>
<tr>
<td>(d) PCL Tube</td>
<td>52 ± 10</td>
<td>4,655 ± 915</td>
<td>0.8471 ± 0.15</td>
</tr>
</tbody>
</table>

Figure 1 Microscopic images of (a) CUPE-S, (b) CUPE-T, (c) nerve autograft and (d) PCL tube in the section of central conduit. (40x)