Keratin Gel as Conduit Filler for Peripheral Nerve Regeneration on a Rat Sciatic Nerve Injury Model

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Introduction
Restoration with sufficient functional recovery after long gap peripheral nerve damage remains a clinical challenge. Incorporation of Glial Cell Line-Derived Neurotrophic Factor double-walled microspheres into poly(caprolactone) (PCL) nerve guides has demonstrated that an off-the-shelf product alternative promotes nerve regeneration. In vitro, keratins derived from human hair enhance the activity and gene expression of Schwann cells. The specific aim of this study was to examine keratin gel as conduit filler for peripheral nerve regeneration in a rat sciatic nerve injury model.

Materials and Methods
PCL nerve guides with GDNF loaded double walled microspheres were prepared. Lewis male rats were divided into three groups: keratin gel filled guides, saline filled guides, and PCL guide with GDNF loaded double walled microspheres only. Under a surgical microscope the sciatic nerve was excised and bridged by a 17 mm nerve guide via 2 transverse mattress sutures. The conduit lumen was filled with normal saline in the positive control group and keratin gel in the experimental group using a 26G needle. As an indicator of functional recovery, gastrocnemius muscle weight, HE and Masson’s stains, and S-100 staining, and PGP9.5 staining were investigated.

Results
As an indicator of functional recovery, gastrocnemius muscle weight was measured. The weight from the saline treatment (74.17±1.32) was lower than keratin group (74.78±2.77), but not significantly. Nerves stained with S-100 and PGP9.5 antibody demonstrated a significantly increased density of Schwann cells and axons in the keratin treated groups compared to the both of saline or empty PCL (control) group (p < 0.05).

Table 1. The area ratio of S-100 protein staining (Schwann cell) and native nerve cross-section.

<table>
<thead>
<tr>
<th>Region</th>
<th>Keratin</th>
<th>Saline</th>
<th>Empty</th>
</tr>
</thead>
<tbody>
<tr>
<td>Proximal</td>
<td>0.9492±0.1345a</td>
<td>0.9329±0.1127a</td>
<td>0.9117±0.1721a</td>
</tr>
<tr>
<td>Proximal</td>
<td>0.8193±0.1112a</td>
<td>0.5773±0.1195b</td>
<td>0.5124±0.1109c</td>
</tr>
<tr>
<td>Middle</td>
<td>0.5246±0.1129a</td>
<td>0.3188±0.0634b</td>
<td>0.2467±0.2055b</td>
</tr>
<tr>
<td>Distal</td>
<td>0.4344±0.0298a</td>
<td>0.2411±0.0902b</td>
<td>0.2407±0.1934b</td>
</tr>
<tr>
<td>Distal</td>
<td>0.3122±0.0210a</td>
<td>0.0938±0.0345b</td>
<td>0.1007±0.1108b</td>
</tr>
</tbody>
</table>

*: means within the same row without the same superscript are significantly different (p < 0.05)

Discussion and Conclusions
PCL-based nerve conduits possess optimal mechanical and degradative properties, rendering them potentially useful in peripheral nerve repair. From our studies, we conclude that PCL nerve guides with GDNF loaded double walled microspheres filled with keratin gel represent a potentially viable guiding material for Schwann cell and axon migration and proliferation in the treatment of peripheral nerve regeneration.

References