EFFECTS OF SCHWANN CELL ALIGNMENT ALONG THE ORIENTED ELECTROSPUN CHITOSAN NANOFIBERS ON NERVE REGENERATION

Introduction
Schwann cell alignment directs neurite outgrowth in the absence of other directional cues in vitro. We have constructed a chitosan nonwoven oriented nanofiber mesh tube with the electrospinning method. The efficacy of this oriented nanofiber mesh on Schwann cell alignment was confirmed by Schwann cells culture, and nerve regeneration after bridge grafting of chitosan nanofiber mesh tube with and without orientation over the nerve gap in the rat sciatic nerve was compared.

Materials and Methods
To fabricate chitosan nanofiber mesh, a positive-charged jet ejected from the chitosan-trifluoroacetic acid (TFA)-methylene chloride (MC) solution was sprayed at the negative-charged collector. The linear rate of the rotating drum was set to 100 rpm to fabricate random nanofiber mesh sheets with a thickness of 0.02 mm, while the linear rate of the rotating drum was set to 1000 rpm. Likewise, the obtained mesh was reeled on a SUS bar with a diameter of 1.2 mm. The completed chitosan mesh tube was composed of randomly arranged nanofibers having an inner diameter of 1.2 mm and an outer diameter of 2.0 mm. To fabricate orinented chitosan nanofiber mesh the linear rate of the rotating drum was set to 1000 rpm. Likewise, the obtained mesh was reeled on the SUS bar with the nanofiber orientation parallel to the axis of the sheet bar to form oriented chitosan mesh tube. The morphology of the surface of the mesh sheet was observed by using scanning electron microscopy (SEM). The cross-section of the mesh sheet was observed by using transmission electron microscopy (TEM). The porosity of the mesh sheet was estimated by the following formula: P = 1 - (mass of dry mesh sheet / mass of a polymer film)

Results and Discussion
The kurtosis of gradient distribution increased in proportion to the linear rate of the rotating drum resulting in improvement of nanofiber alignment. As a result of fiber orientation of the mesh sheet, the tensile strength parallel to the axis of the sheet increased compared with non-oriented fiber sheet. Furthermore, because Schwann cells cultured on the chitosan mesh aligned along the nanofibers, oriented fibrous sheets facilitate Schwann cell column formation.

Function recovery, first sensory nerve followed by motor nerve function, and electrophysiological recovery occurred in time in the oriented group approximately matched those in the isograft. Furthermore, histological analysis revealed that nerve regeneration through the chitosan mesh tube with nanofiber orientation compares favorably with that of isograft. Electrospun nanofibrous structures can promote cell growth and provide extensive surface area for cell attachment because of their extracellular matrix like three-dimensional structure possessing high surface area-to-volume ratio. Moreover, in the oriented chitosan nanofiber mesh tube, Schwann cells that migrate into the lesion area from the amputated nerve stumps are promoted to create a Schwann cell column. This mechanism may result in a marked restoration of nerve function after bridge grafting over the peripheral nerve gap.

<table>
<thead>
<tr>
<th>STSF</th>
<th>non-oriented</th>
<th>oriented</th>
<th>*p&lt;0.05 isograft</th>
</tr>
</thead>
<tbody>
<tr>
<td>5w</td>
<td>0.582 ± 0.070</td>
<td>0.516 ± 0.051</td>
<td>0.504 ± 0.028</td>
</tr>
<tr>
<td>10w</td>
<td>0.594 ± 0.111</td>
<td>0.492 ± 0.079</td>
<td>0.450 ± 0.045</td>
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<tr>
<td>15w</td>
<td>0.526 ± 0.025</td>
<td>0.470 ± 0.042</td>
<td>0.426 ± 0.035</td>
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<tr>
<td>20w</td>
<td>0.483 ± 0.055</td>
<td>0.424 ± 0.035</td>
<td>0.414 ± 0.036</td>
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</tbody>
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von Frey test:
The recovery of motor and sensory function associated with the sciatic nerve, von Frey hair test and static toe spread factor (STSF) was evaluated, respectively, every 5 weeks until 30 weeks post-implantation.

Electrophysiological evaluations were carried out 30 weeks after implantation (N=5 in each group). A bipolar stimulating electrode was placed at the sciatic notch, and compound muscle action potentials were recorded on the triceps surae muscle. The results were assessed by the following formula: amplitude or latency in the experimental side / amplitude or latency in the normal side.

<table>
<thead>
<tr>
<th>number of axon</th>
<th>6193 ± 1248</th>
<th>13188 ± 2658</th>
<th>16488 ± 3342 **</th>
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</thead>
<tbody>
<tr>
<td>axon diameter (µm)</td>
<td>1.66 ± 0.19</td>
<td>2.06 ± 0.31</td>
<td>2.09 ± 0.37</td>
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<td>axon area (µm²)</td>
<td>37969 ± 16548</td>
<td>78617 ± 15134</td>
<td>87028 ± 33736 **</td>
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<tr>
<td>nerve area (µm²)</td>
<td>220250 ± 49089</td>
<td>302250 ± 76526</td>
<td>359000 ± 29232 **</td>
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