

## PERIPHERAL NERVE RECONSTRUCTION USING ACELLULAR NERVE GRAFTS

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RECONSTRUCTIVE OPTIONS for peripheral nerve gaps are currently limited. Autogenous nerve grafts continue to be the "gold standard," but donor site morbidity precludes the harvest of all but a few sensory nerves. Investigational methods include allografts, which require immunosuppression,<sup>1</sup> and various biologic and synthetic guidance channels.<sup>2</sup> Acellular nerve and muscle grafts also have been tried as potential conduits, but nerve regeneration beyond a 2-cm gap has not been consistently observed. This seems to be related to the absence of Schwann cells, known to be essential for successful nerve regeneration.<sup>3</sup> As a first step toward engineering a nonimmunogenic composite graft system, we have developed a chemical acellularization method for peripheral nerves that yields an immunologically inert conduit (unpublished results). We hypothesize that acellular nerve grafts made

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### Results

	n	Body mass (mg)	EDL mass (mg)	IS at 15 wk	F <sub>0</sub> (mN)	sF <sub>0</sub> (kN/m <sup>2</sup> )
Cell 2-cm	5	412 ± 32	114 ± 11	-0.04 ± 0.08	1,790 ± 315	188 ± 34
Cell 4-cm	5	401 ± 29	112 ± 6	0.05 ± 0.15	1,980 ± 695	216 ± 80
Acell 2-cm	4	393 ± 28	73 ± 23*	-0.20 ± 0.20	726 ± 608*	106 ± 65*
Acell 4-cm	5	379 ± 22	33 ± 3†	-0.34 ± 0.13†	6 ± 6†	2 ± 2†

Data are displayed as mean ± SD. Cell indicates cellular graft (autograft); Acell, acellular nerve graft; EDL, extensor digitorum longus muscle; IS, intermediate toe spread index; F<sub>0</sub>, maximum tetanic isometric force; and sF<sub>0</sub>, specific force. \*Statistically significant at  $P < .05$ , Cell 2-cm vs Acell 2-cm; 2-way analysis of variance (ANOVA). †Statistically significant at  $P < .05$ , Cell 4-cm vs Acell 4-cm; 2-way ANOVA.

using our technique can support nerve regeneration across a 2-cm nerve gap in the rat, but that this conduit alone will not support regeneration over a critical 4-cm gap.

### MATERIALS AND METHODS

Peroneal nerves were harvested from adult male Fischer-344 rats and acellularized using a technique developed in our laboratory. In 19 isogenetic rats, nerve gaps of 2 or 4 cm were created in the left peroneal nerve. This gap was repaired with either the native nerve segment (Cell groups) or an identical length of acellular graft (Acell groups). Walking tracks were used to evaluate integrated motor function preoperatively and at 3-week intervals postoperatively. Specifically, the intermediate toe spread (distance between toes 2 and 4) was measured bilaterally and used to calculate an intermediate toe spread index (IS).<sup>4</sup> Fifteen weeks postoperatively, maximum tetanic isometric force (F<sub>0</sub>) of the left extensor digitorum longus (EDL) muscle was measured, and specific force (sF<sub>0</sub>) was calculated, normalizing F<sub>0</sub> to the muscle cross-sectional area.

### RESULTS

Mean data for each group are shown in the Table. Significant differences ( $P < .05$ ) were observed for EDL muscle mass, F<sub>0</sub>, and sF<sub>0</sub> between Cell and Acell rats at both gap lengths. The IS, derived from walking track measurements, was significantly depressed in the Acell 4-cm group compared with the Cell 4-cm group. Statistical analysis with 2-way analysis of variance revealed the most significant factor accounting for the differences seen was the cellularity of the graft.

Histologic examination of muscle sections confirmed the presence of healthy reinnervated muscle in all but the Acell 4-cm group, which appeared chronically denervated. Examination of sections from the distal end of the 4-cm acellular grafts failed to identify any axons crossing this gap. In contrast, sections of distal graft from all other groups revealed a multitude of axons, many of which are myelinated.

### DISCUSSION

At the present time, nerve autografting remains the standard of care for repair of peripheral nerve gaps. However, the search for an alternative conduit is an area of intense focus for many investigators. We have developed an acellularization technique that preserves the delicate ultrastructure of peripheral nerve while removing the most antigenic components, namely Schwann cells and myelin. By directly quantifying end-organ recovery of function, we have clearly demonstrated that these acellular nerve grafts can support nerve regeneration over a short 2-cm

gap. At this gap length, walking tracks confirm partial recovery of integrated function.

At the 4-cm gap length, we saw complete failure of reinnervation, as evidenced by walking tracks, histology, and absence of any force-generating capability in the muscle. This is consistent with the existing literature and provides us with an appropriate negative model against which to compare future studies. In conclusion, these data support our hypothesis that although acellular nerve grafts, prepared by our technique, can support nerve regeneration across a 2-cm gap, the extracellular matrix alone cannot support regeneration across a critical 4-cm nerve gap.

#### REFERENCES

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