Long Term Stability of Regenerative Peripheral Nerve Interfaces (RPNI).

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Purpose: Our goal is to interface amputee peripheral nerves to engineered systems for neuro-prosthetic limbs. Two forms of neurotized regenerative peripheral nerve interface (RPNI) were compared for long term stability in the rat hind limb amputation model. RPNIs contained either cultured autogenous myoblasts or freely transferred autologous muscle. We examined tissue integrity and functional muscle reinnervation at least 12 months after RPNI neurotization.

Methods: An RPNI (15x5 mm) was implanted in each rat hind limb. RPNIs were constructed with either: cultured myoblasts (MyoCells) n=6, or freely transferred muscle (MusTran) n=9, which were surrounded by decellular submucosa. Each RPNI was neurotized with the transected proximal peroneal nerve stump. Twelve months following implantation, the RPNI's were functionally assessed for compound muscle action potentials and nerve electrophysiologic outcome measures. Non-operated rats were tested as controls.

Results: At harvest, all RPNIs were covered by a thin, fibrous capsule less than 1mm in thickness. All RPNIs were well vascularized and without evidence of infection. MusTran RPNIs' muscle fibers regenerated to 33% cross sectional area (CSA) of control CSA; while MyoCell RPNI fibers matured to reach only 3%. Peroneal nerve electrodiagnostics showed MusTran RPNI performance was similar to Control for compound muscle action potential (CMAP) latency, 70% CMAP amplitude, and 75% CMAP area (Table 1). MyoCells RPNI compared with MusTran RPNI recovered 6% CMAP area and were 50% slower. Neuromuscular junction (NMJ) staining indicated extensive muscle fiber reinnervation on MusTran RPNI muscle fibers while MyoCells RPNI showed few neuromuscular junctions (NMJ) (Fig 1).

Conclusion: Within RPNIs, freely transferred muscle or cultured myoblasts, when neurotized by transected peroneal nerve, became reinnervated. Vital muscle, nerve fibers, and NMJ were supported for up to 12 months in both the MusTran RPNIs and the MyoCells RPNIs. When nerve and muscle functions were compared with controls, RPNIs containing neurotized free muscle transfers were superior to those containing cultured myoblasts.

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Table 1. Comparisons for Control & Regenerative Peripheral Nerve Interface Groups.

	Dependent Variable	Groups: Control	MyoCells	MusTran
Nerve Conduction to RPNI.	Number in each group	37	6	9
	CMAP Amplitude, mV	18.8 ± 8.1	0.4 ± 0.5*	13.2 ± 11.0*,†
	CMAP Latency, ms	1.4 ± 0.3	2.6 ± 2.7*	1.5 ± 0.3†
	CMAP Area, mVms	20.5 ± 9.8	0.9 ± 0.8*	15.4 ± 12.6
	CMAP Duration	4.7 ± 6.1	2.3 ± 3.3	1.9 ± 0.2
	Conduction velocity, M/s	24.7 ± 4.6	14.5 ± 10.2*	19.7 ± 6.8
	Stimulation, mA	2.8 ± 8.1	2.7 ± 8.1**	17.1 ± 14.4*
	Stimulation duration, ms	0.7 ± 0.8	0.4 ± 0.5	0.1 ± 0.0
Histo-logy	Muscle Fiber CSA, mm^2	2151 ± 73 (n=4)	65 ± 95* (n=2)	704 ± 386*,† (n=6)

Compound Muscle Action Potential (CMAP); Cross sectional area (CSA). Significant results indicated by: *less function than Control; † better than Myoblast Group, ** better than Muscle Transfer. α≤0.05. mV=millivolt, ms=millisecond, M=meter; mA=milliAmp.

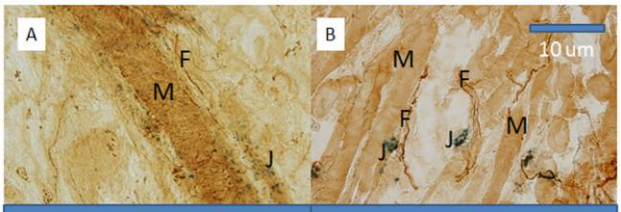

Figure 1. Longitudinal sections through RPNI. Blue stains indicate neuromuscular junctions (NMJ). Brown lines are nerve fibers. M, Muscle Fiber; F, Nerve Fiber; J, Neuromuscular junction.