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NICOTINIC RECEPTORS MODULATED CONTRACTILE ACTIVITY AND COMPLIANCE OF MUSCLE-DERIVED STEM CELLS INCORPORATED INTO ACELLULAR SCAFFOLDS FOR URETHRAL AND BLADDER RECONSTITUTION

Aims of Study
To explore the contractile activity and biomechanical properties of muscle-derived stem cells (MDSC) incorporating into small intestinal submucosa (SIS) scaffolds for bladder and urethral reconstitution.

Methods
After harvesting hindleg muscle from mice, MDSC were isolated using the pre-plate technique. The late plate cell population were transduced with retrovirus-LacZ gene and then suspended in Hank's balanced salt solution for use. MDSC were placed into single layer SIS cell culture inserts. Twenty-five different preparations of MDSC and/or SIS were then incubated at 37 ?, for either 10 or 20 days. LacZ staining and Masson-Trichrome staining were performed to reveal MDSC and SIS. Immunohistochemical staining for the muscle type acetylcholine receptor (AChR), Hoechst staining for nuclei and Desmin staining for myoblasts/myotubes were also performed. The histochemical properties of MDSC were examined after different times in culture. Using the biaxial testing method, the tissue deformations were measured continuously using real-time video marker tracking. Areal strain was calculated to quantify the compliance of the specimens. Then, the data obtained were compared between different groups. Fifty additional preparations of SIS and MDSC were incubated at 37^0C, for 1, 4 and 8 weeks, respectively. MDSC/SIS were examined histologically and also mounted in a bath and the property of the isometric contractions was measured. Electrical field stimuli, succinylcholine, carbachol, KCl, Ca^{++}- free Krebs solution with EGTA and distilled water were applied into the bath sequentially.

Results
Histologically, LacZ, and Masson-Trichrome staining revealed MDSC could migrate and distribute throughout the SIS and form myotubes. The mean (± S.E.) areal strain in the control group (n=4) of the non-incubated SIS was 0.182 ± 0.027 (n=5). After 10 days incubation, the mean (± S.E.) areal strain in MDSC/SIS (0.247 ± 0.014, n=5) was not significantly different (P = 0.142) from that of incubated SIS alone (0.200 ± 0.024, n=6). After 20 days incubation, the mean (± S.E.) areal strain of MDSC/SIS (0.255 ± 0.019, n=5) was significant higher (P = 0.027) than that of incubated SIS alone (0.170 ± 0.025, n=5). The biaxial testing results exhibited the classic biological non-linear stress-strain response. The specimens were in a state of nearly pure biaxial strain with negligible shear.
Multiple staining for AChR, Hoechst and Desmin demonstrated AChR expression in MDSC/SIS preparations after 4- and 8-week incubations but not after 1-week incubations. In MDSC/SIS, spontaneous contractile activity (SCA) was noted in 4 -week (5/6 specimens) and 8-week cultures (8/8 specimens) but not in 1-week cultures (N=11). All SIS control groups after 1 (N=11), 4 (N=6) and 8-week (N=8) incubation did not show any activity. In 4-week MDSC/SIS, the frequency and amplitude of SCA were decreased in 2/5 specimens by succinylcholine 10 uM and 5/5 specimens by succinylcholine 20uM. In 8-week MDSC/SIS, the frequency and amplitude of SCA were decreased in 8/8 specimens by succinylcholine 10 uM and 20 uM. Electrical field stimulation, carbachol and KCl did not alter the frequency, amplitude and pattern of the SCA in MDSC/SIS. SCA was blocked by Ca^{++}-free Krebs solution with EGTA 200 μM or distilled water.

Conclusions
MDSC can be incorporated into SIS forming myotubes capable of contracting. MDSC/SIS exhibited better compliance than incubated SIS alone in 20 days preparations. The histochemical demonstration of AChR expression in MDSC/SIS preparations supports the

view that AChRs are responsible for contractile activity in MDSC/SIS. The contractile activity of this 3-dimensional construct is Ca^{++} dependent and modulated by nicotinic receptors. MDSC seeding of acellular matrix may become a functional sling to reengineer the deficient sphincter or as contractile bladder augmentation.