Mitigation of hypertrophic scar contraction and stiffening via an elastomeric biodegradable scaffold

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Statement of Purpose: Hypertrophic scar (HSc) occurs in 40-70% of patients treated for third degree burn injuries. Current burn therapies rely upon the use of bioengineered skin equivalents (BSEs), which assist in wound healing but do not prevent HSc contraction. HSc contraction occurs of 6-18 months and results in the formation of a fixed, inelastic skin deformity that causes severe pain, disfigurement, and loss of motion across joints, with 60% of cases occurring across a joint. We propose to develop BSEs that can persist throughout the remodeling phase of repair. In this study we investigate the impact of a degradable and elastomeric poly(l-lactide-co-ε-caprolactone) (PLCL) on HSc contraction.

Methods: Electrospinning was used to generate randomly aligned, PLCL scaffolds with an average fiber diameter of 6μm. After surface treatment with bovine type I collagen, the collagen coated PLCL (ccPLCL) scaffolds were characterized for tensile and fatigue properties, and compared to standard of care BSE (Integra™), human skin, and human scar tissue. ccPLCL scaffolds were surgically inserted beneath skin grafts in a validated immune-competent murine HSc contraction model for four, eight, and sixteen weeks, with comparison to skin graft alone or Integra™. Tensile testing was carried out on uninjured mouse skin as compared to scar tissue harvested from mice treated with ccPLCL, Integra, and skin graft alone. Degradation analysis of explanted ccPLCL scaffolds was evaluated by GPC and NMR.

Results: The elastic moduli of ccPLCL (8.3±0.67 kPa) and Integra (6.5±0.91 kPa) were significantly less than human skin (17±1.6 kPa), and scar (55±14 kPa). The ultimate tensile stress for ccPLCL scaffolds (1.3±0.17 GPa), human skin (2.6±0.040 GPa), and human scar (2.7±0.50 GPa) were significantly greater than that of Integra (0.26±0.020 GPa). In contrast, the elongation at break for ccPLCL (1300±170 kPa) scaffolds was significantly higher than the values obtained for Integra (75±4.4 kPa), human skin (200±16 kPa), and human scar (140±16 kPa). The storage modulus, loss modulus, and tanΔ of ccPLCL scaffold subjected to 10% strain at 1Hz showed negligible deterioration over 24h, or 15,000 cycles.

All data presented from animal studies are described in terms of percentage of original wound size, where 100% is the wound size at d0, and a fully contracted wound is 0% of its original size. All samples were applied beneath a split-thickness skin graft. Murine wounds treated with skin grafts alone contracted to 47±2.0% at d30, while wounds treated with Integra™ contracted to 28±1.8% (Fig. 1A). In contrast, wounds treated with ccPLCL scaffolds showed significantly decreased contraction, down to 95±5.8% at d30 (Fig 1A). d30 ccPLCL explants exhibited an elastic modulus (5.7±2. kPa), significantly lower than tissue alone in both mouse skin (36±3.3 kPa) and scar (80±17 kPa) samples, although not significantly different from that prior to implantation (9.7±0.20 kPa). The molecular weight of the scaffolds decreased by 49% from M_n=151 kDa to 73.2±6.5 kDa over the implanted period. NMR analysis shows that the lactide (LA) moiety was noticeably more rapidly decreased than the caprolactone (CL) units. The mole fraction of LA decreased from 50% to 44% in 30 days, while that of CL increased from 50% to 56%. Studies on the long-term effect of scaffold degradation on HSc contraction are ongoing.

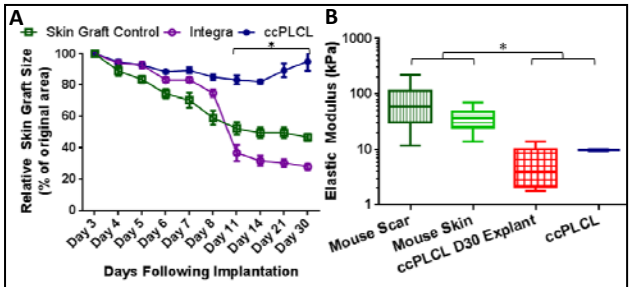

Table 1	Mn (kDa)	Mn (%)	Mw (kDa)	Mw (%)	PDI	LA:CL
Initial	151	100.0	298	100.0	1.9	50:50
d30 Explant	73	48.6	135	50.7	2.0	44:56

Figure 1. (A) ccPLCL treated wounds contracted significantly less than Integra or control wounds. (B) ccPLCL treated skin grafts were significantly less stiff uninjured mouse skin and mouse scar (skin graft treated wounds) at d30. (C) GPC and NMR analysis revealed decreasing number average (M_n) and weight average (M_w) molecular weight, increased polydispersity index (PDI) and altered LA:CL ratio.

Conclusions: PLCL scaffolds displayed appropriate elastomeric and tensile characteristics for implantation beneath a human skin graft. HSc contraction was significantly greater in animals treated with standard of care, Integra™, as compared to those treated with ccPLCL scaffolds. Wounds treated with ccPLCL were significantly less stiff than control wounds at d30 in vivo. Degradation of PLCL at d30 in vivo was evidenced by decreasing M_n, increasing PDI, and altered CL:LA ratio. These data suggest that scaffolds which persist throughout the remodeling phase of repair may represent a clinically translatable method to prevent HSc contraction.