Diaphragmatic Reconstruction by Electrospun Aligned Fibrous Scaffolds

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Statement of Purpose: Abnormal development of the diaphragm causes a life-threatening problem for the newborn. The surgical treatments for large diaphragmatic defect are required. A variety of materials have been reported for diaphragmatic repair in the infants born with diaphragmatic defect, including the use of prosthetic materials [1]. However, the repairing patches with these prosthetic materials do not grow with the child and may possibly cause granulation, restricted chest wall development, patch separation, and recurrent herniaton. We have previously shown that the electrospun poly(ε-caprolactone) (PCL)/collagen scaffolds provided unidirectional fiber orientation that can guide cell alignment and enhance myotube formation [2]. In this study we examined the feasibility of using the aligned electrospun PCL/collagen fibrous scaffolds for diaphragmatic muscle reconstruction.

Methods: Scaffolds composed of PCL and type I collagen were fabricated using electrospinning techniques (Figure 1a and 1b). Twenty three rats underwent left subcostal laparotomy, left central hemi-diaphragmatic excision (2 × 3 cm; approximately 70%) and reconstruction with an aligned fibrous scaffold (Figure 1c). Twenty nine normal rats received sham operation only. Radiographic and magnetic resonance imaging (MRI) analyses were performed at each time point. Blood gas concentrations, including CO_2, O_2, and pH, and body weight were also measured. The diaphragm specimens were retrieved at their predetermined time points (1, 2, 4, and 6 months) for histological and immunohistological analyses and mechanical test.

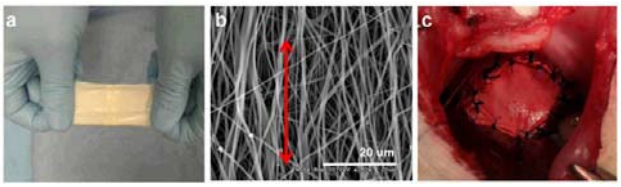

Figure 1. (a) Gross appearance and (b) SEM image (×2.0K) of the electrospun aligned PCL/collagen fibrous scaffolds. (c) The scaffolds were implanted in the left side diaphragm of the rat.

Results: All animals survived without signs of infection or rejection. There was no evidence of chest wall deformity or other changes (Figure 2). Radiographic and MRI analyses showed no evidence of herniation or eventration. At the 1 and 2 month time points, blood gas analyses revealed that O_2 concentration was lower and CO_2 and pH were higher in experimental rats after surgery (P<0.05); however, these measurements returned to near control levels at the 3 and 4 month time points (P<0.05). Scaffold implanted rats had lower body weights at the 1 and 2-month time points (P<0.05). However, the experimental rats gained weight at the 4 and 6 month time points and appeared to have similar growth rates to those observed in the control rats (P<0.05). At retrieval, all engineered diaphragms were intact without necrosis, hernia or retraction. Histological and immunohistochemical evaluations revealed muscle ingrowth and vascularization of the scaffolds. The mechanical properties of the scaffolds tended toward that of normal diaphragm at the designated time points (Figure 3). Our results show that the aligned electrospun scaffolds allowed muscle cell migration and tissue formation.

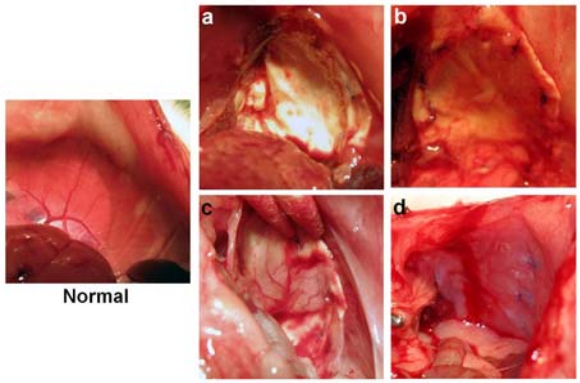

Figure 2. The normal diaphragm and the implanted scaffolds at (a) 1, (b) 2, (c) 4, an (d) 6 months after the implantation. There is more vascularity in the implanted scaffold from later time points, and no visible necrotic area in the repairing sides.

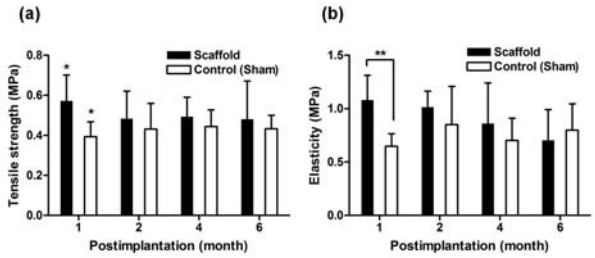

Figure 3. Tensile properties of the implanted scaffolds with time; (a) tensile strength and (b) elasticity (*P<0.05 compared to control scaffold and **P<0.05).

Conclusions: Diaphragmatic reconstruction with electrospun aligned PCL/collagen scaffold leads to good structural and functional outcomes. The novel scaffold that works as a vehicle to induce *in vivo* tissue engineering process might be a potential material for surgical repair of large diaphragmatic defect. Further investigation in a larger animal model with a longer period will be necessary.

References:

[1] Conconi MT et al. J Biomed Mater Res. 2009;89:304-316.
[2] Choi JS et al. Biomaterials. 2008;29:2899-2906.