Biomaterial Induced Host Stem Cell Recruitment for *In Situ* Muscle Tissue Regeneration

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Introduction

In this study we sought to utilize muscle satellite/ progenitor cells residing in host muscle to regenerate muscle tissue using a target-specific scaffold. The objectives were to evaluate various signaling molecules (myogenic factors) for muscle cell migration, proliferation, and differentiation *in vitro* and to investigate the feasibility of the use of myogenic factor-incorporated scaffolds to enhance host muscle satellite/progenitor cell mobilization and recruitment *in vivo*.

Materials and Methods

Nonwoven poly(L-lactic acid) (PLLA Scaftex®; density 43 mg/cc, thickness 4 mm) scaffolds (4×5×10 mm^3) were implanted in the gluteus maximus muscle of SD Rats. The implanted scaffolds were retrieved at 1, 2, 3 and 4 weeks after implantation and characterized by H&E and immunohistochemistry with muscle satellite/progenitor specific makers (Pax3, Pax7 and MyoD). To evaluate myogenic factors that affect primary muscle cells, we selected a series of growth factors (HGF, SDF, bFGF, FGF-6, EGF, IGF-1, and IGF-2) and applied to muscle cells to determine the levels of cellular migration, proliferation, and differentiation *in vitro*. To evaluate the feasibility of the use of myogenic factors *in vivo*, we fabricated heparin-immobilized gelatin scaffolds (Gelfoam™) containing each myogenic factor. The myogenic factor-incorporated scaffolds were implanted in the gluteus maximus muscle of rats and were retrieved at 1 and 2 weeks after implantation. The retrieved samples were characterized by immunohistochemistry with muscle satellite/progenitor specific marker (Pax7).

Results

Nonwoven PLLA scaffolds provided high porosity and uniform pore structure with long-term degradation rate. The retrieved scaffolds showed a progressive tissue ingrowth over time [Fig. 1 (top)]. By the fourth week, the scaffolds were completely infiltrated by host cells, including immune/inflammatory cells and stromal-like cells. Fig. 1 (bottom) shows that host cells expressed Pax7 within the implanted scaffolds at all time points. These findings indicate that host muscle satellite/progenitor cells are able to migrate into the implanted scaffolds. In addition, the myogenic factors have effectively promoted muscle cell migration, proliferation, and differentiation *in vitro* and cells expressing Pax7 were increasingly mobilized into the implanted biomaterials containing myogenic inducing factors (Fig. 2).

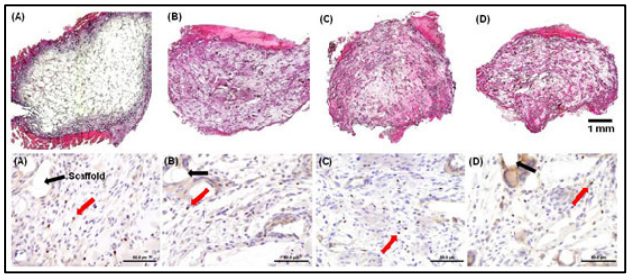

Fig. 1. H&E staining (top) and immunohistochemistry for Pax7 (bottom) of the retrieved scaffolds at (A) 1, (B) 2, (C) 3, and (D) 4 weeks after implantation (black arrow: remaining scaffolds, red arrow: Pax7 expressed cells).

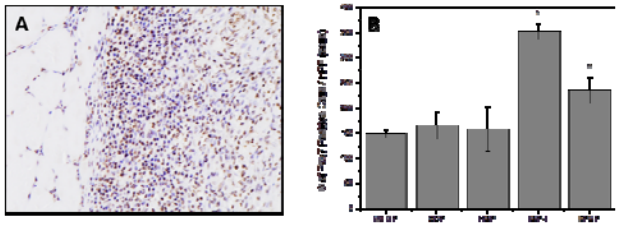

Fig. 2. Immunohistochemistry for Pax7 shows positive expression of host cells in the IGF-I loaded gelatin scaffold retrieved at 2 week post implantation (A). The number of Pax7 positive cells per high power field (HFP) (B).

Discussion and Conclusions

This study suggests that it may be possible to use the body's biologic and environmental resources for *in situ* tissue regeneration. We demonstrate that cells expressing muscle satellite/progenitor cell markers can be mobilized into an implanted biomaterial and that these cells are capable of differentiating into muscle cells. Therefore, it may be possible to enrich the infiltrate with such cell types and control their fate, provided the proper substrate-mediated signaling can be imparted into the scaffold. Thus, *in situ* regeneration of functional muscle tissue through host cell recruitment may be possible.

Acknowledgments

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