Biomaterial Induces Host Stem Cell Recruitment for *In Situ* Muscle Regeneration

Young Min Ju, Chang Mo Hwang, Anthony Atala, James J. Yoo, and Sang Jin Lee
Wake Forest Institute for Regenerative Medicine, Wake Forest University Health Sciences, Winston-Salem, NC 27157, USA

Statement of Purpose: In this study we aim to utilize stem or progenitor cells residing in the host to regenerate muscle tissue through the use of a target specific scaffold. This approach is based on the demonstration that almost every tissue in the body contains some type of stem or progenitor cells. The putative healing mechanisms and classic foreign body reaction to implanted biomaterials have also been characterized. However, these two mechanisms would seem to be in conflict with one another, particularly with respect to functional outcome. While small, localized day-to-day injuries are regenerated by the body's stem and progenitor cell machinery, large traumatic injury overwhelms this system and survival mechanisms take over. This process often creates a deficit of functional recovery. The objectives of this study are to investigate this possibility using an animal model to initiate cell mobilization, recruitment, and differentiation *in vivo* and to demonstrate the muscle tissue regeneration.

Methods: In order to evaluate the host cell infiltration to an implanted biomaterial in a rodent model, we selected poly(L-lactic acid) (PLLA) mesh (Scaftex®; density 43 mg/cc, Biomedical Structures, Inc.) as a scaffold to accommodate host cell infiltration. PLLA scaffolds ($5\times10\times4$ mm^3) were implanted in the gluteus maximus muscle site of CD1 mice. The implanted scaffolds were retrieved at 1, 2, 4, and 6 weeks after implantation. The retrieved samples were characterized by histochemistry, immunohistochemistry, and western blotting. Cells residing within the biomaterials were isolated by enzymatic digestion. The cells were resuspended in culture medium, plated on tissue culture dishes, and grown to confluence for 2–3 weeks at 5% CO_2, 95% humidity, and 37°C. The isolated infiltrating cells were analyzed by florescence-activated cell sorting (FACS) and immunocytochemistry for various stem cell markers.

Results: Figure 1 showed that host cells were vigorously increased at 1 week post implantation and then progressively decreased over time. By the fourth and sixth week, host cells were dominantly accumulated around the individual scaffold fibers and abundant vasculature was present within the scaffold. Masson's trichrome staining of representative sections after implantation showed the gradual buildup of extracellular matrix proteins.

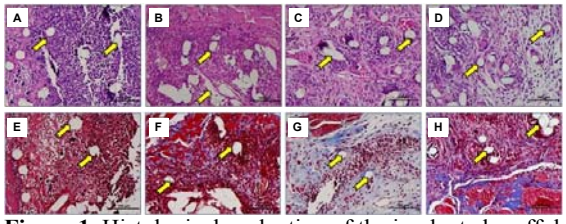

Figure 1. Histological evaluation of the implanted scaffolds. (A-D) H&E and (E-H) Masson's trichrome staining at (A,E) 1, (B,F) 2, (C,G) 4, and (D,H) 6 weeks after implantation. Arrows indicated the fibers of the implanted scaffold.

Figure 2 showed that anti-Pax7 was expressed within the implanted biomaterials with all time points. These findings indicate that host muscle stem/progenitor cells are able to migrate/recruit into the implanted biomaterials. Western blot analysis showed that cells expressing Pax7 are present within the implanted biomaterials and further supports our immunolocalization results (Figure 2D).

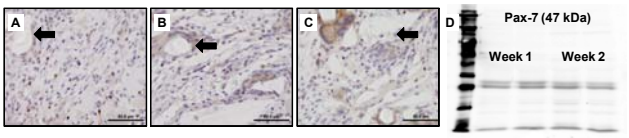

Figure 2. Immunohistochemistry for Pax7 expression with retrieved scaffolds at (A) 1, (B) 2, and (C) 4 weeks after implantation. Arrows indicated the scaffold fiber mesh. (D) Western blotting of the retrieved biomaterials. ECM proteins extracted from the retrieved biomaterials were size-separated by SDA-PAGE and detected Pax7.

The isolated infiltrating cells grew in culture and were expanded. FACS analyses of the cultured cells failed to express endothelial progenitor cell markers, hematopoietic stem cell markers, and mesenchymal stem cell markers, CD31, CD34, CD44, CD117, and CD133. However, the cells showed a strong expression of muscle stem/progenitor cell markers, including Pax3, Pax7 and myoD, but did not express desmin. Immunofluorescent staining of the isolated host cells confirmed the expression of Pax7 and myoD (Fig. 3).

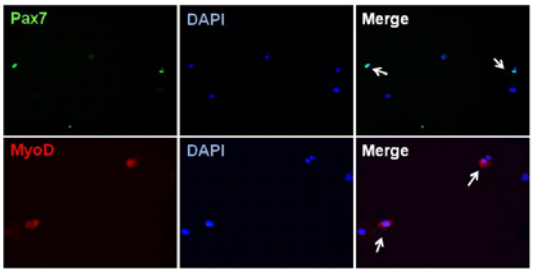

Figure 3. Immunofluorescent staining for Pax7 and myoD of the isolated infiltrating host cells. This indicates that host muscle stem cells can be recruited into the implanted biomaterials.

Conclusions: In this study we investigated the possibility of using the body's biologic and environmental resources for in situ tissue regeneration. We show that cells expressing muscle stem/progenitor cell markers are also mobilized into the 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 for in situ regeneration of functional muscle tissue through host cell recruitment.

References:

Lee SJ et al. Rejuvenation Res. 2008;11:747-756.

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