INTRODUCTION
The emerging field of tissue engineering represents a new approach to the repair of diseased or damaged tissues, particularly when donor tissue is insufficient and/or unavailable. For the treatment of muscle loss from traumatic injury, tumor ablation, vascular insult, or degenerative muscle disease, only few alternatives currently exist for the restoration of damaged muscle tissues. For skeletal muscle tissue engineering to succeed, an appropriate autologous cell source needs to interact with a biocompatible and biodegradable scaffold that is capable of mimicking the structure and functions of the natural extracellular matrix environment. Our previous work has shown that electrospun, three-dimensional nanofibrous structures share morphological similarities to collagen fibrils, and are capable of promoting favorable biological responses from seeded cells [1,2]. The present study investigated the potential of an electrospun three-dimensional nanofibrous scaffold for the in vitro engineering of human skeletal muscle, by examining the effect of fiber alignment on the proliferation and differentiation of human skeletal muscle myoblasts (hSKMM).

METHODS
Preparation of Poly(L-Lactic Acid) (PLLA) nanofibrous scaffolds
Aligned and nonaligned electrospun nanofibrous scaffolds were created using methods previously described [3]. Since initial results enhanced cell adhesion to nanofibers when the biomaterial was coated with human plasma fibronectin at a concentration of 10 µg/cm², constructs were incubated in fibronectin solution for 48 h at 4°C prior to cell seeding.

Myoblast-nanofiber constructs
hSKMM (Cambrex, MD) were expanded in monolayer culture to obtain sufficient cells for seeding. P3 cells were seeded at a density of 50,000 cells/cm² onto 0.5 cm² PLLA constructs (thickness = 1 mm).

MTS Cell Proliferation Assay
Cell proliferation was assayed using the MTS kit at culture Day 0 (5 hours), Day 7, Day 14, and Day 21. At each time point, constructs (n=5) were incubated in MTS assay kit for 60 minutes, after which the eluates were analyzed spectrophotometrically at 490 nm.

Immunofluorescence Staining
To assess the cellular maturation and differentiation, the cultured constructs were harvested at Day 0 (5 hours), Day 7, Day 14, and Day 21, and cells stained with the Hoechst fluorescent nuclear stain, and with a mouse anti-myosin heavy chain antibody.

RESULTS
The time course of cell proliferation in aligned PLLA nanofibers and nonaligned nanofibers is shown in Figure 1. At D0 of culture, equal number of cells attached to the two constructs. However, by D7, a higher rate of proliferation of hSKMM was seen in the nonaligned fiber construct, a pattern that continued at all subsequent time points. In both constructs, peak cell number was reached by D14. After this point, cell proliferation decreased, most likely due to cell differentiation into myotubes.

Immunofluorescence staining of hSKMM (Fig. 2) grown on aligned and non-aligned PLLA nanofibers show the presence of small amorphous cells at D0. By D7 these cells took on an elongated myoblastic morphology, eventually by D14 reaching a state of high cell number and a more spindle shaped myoblastic morphology. By D14, the appearance of multinucleated myotubes was observed. These results are consistent with the MTS data, indicating that cells were proliferating for the first D14 until adequate cell number and cell contact were achieved, leading to the differentiation of the cells into myotubes.

DISCUSSION
We have successfully cultured hSKMM on aligned and non-aligned electrospun three-dimensional PLLA nanofibrous scaffolds. These scaffolds provide a substrate for the proliferation and differentiation of hSKMM, a three-dimensional guide for tissue formation. As previously reported [1,2], these fibers provide a number of advantages for cell based tissue engineering, including increased porosity, wide range of pore size distribution, increased surface area to volume ratio, fiber diameter that is similar to that of native collagen fibrils, and increased mechanical properties, and represent a promising biomaterial for skeletal muscle tissue engineering. In this study, by aligning the nanofibers, a scaffold structure more similar to the native architecture of skeletal muscle tissue is fabricated. We are currently analyzing the gene expression and protein profiles of hSKMM cultured on the aligned and non-aligned PLLA nanofibers, and examining cellular ultrastructure using scanning electron microscopy, to evaluate the efficacy of skeletal muscle tissue engineering using this approach.

REFERENCES
1) Li+ JBMR 2003: 67A:1105-14; 2) Li+ Biomaterials 2005: 26: 599-609; 3) Li+ 5th ORS Combined Meeting