A Collagen-Based Matrix that Enhances Cardiac and Skeletal Myogenesis in Mouse Embryonic Stem Cells

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Statement of Purpose: It has been shown previously that a collagen matrix containing sialyl Lewis X (sLe^x), an L-selectin ligand found on CPCs, can enhance the regeneration of ischemic muscle[1], and accelerate the differentiation of C2C12 myoblasts. In this study, we tested whether or not these matrices would also support the formation of a myogenic progenitor population from mouse embryonic stem cells (ES). This population should replenish the satellite cell niche when engrafted into muscle. Considering the difficulty of grafting stem cells *in vivo*, a matrix that can support the formation of myogenic progenitor cells would be valuable as a potential method of delivery for future cell therapy approaches. The effects of a collagen matrix, with and without sLe^x, were evaluated during skeletal and cardiac myogenesis in mouse ES cells.

Methods: mES cells were cultured on plates coated with collagen or sLe^x-collagen matrix and RNA and protein were harvested at 9, 12, 15 and 20 days for the analysis of skeletal muscle markers by Q-PCR and western blots. Expression of cardiac muscle markers was also analyzed on each surface, on days 10 and 15 of differentiation[2]. Immunofluorescence staining for Myosin heavy chain (MHC), structural genes such as alpha-actinin and muscle regulating transcription factors such as Pax3 and Pax7 was performed. All the results were compared to control tissue culture plates, which is a surface with no coating.

Results:
Skeletal muscle: Immunofluorescent staining identified skeletal myocytes on the collagen surface with a higher number of nuclei compared to the control surface, indicating enhanced cell fusion. This was confirmed by Q-PCR analysis of a skeletal muscle-specific fusion marker, nfatc3. Q-PCR analysis of skeletal muscle precursor markers showed a significantly higher expression of Pax3 and Pax7 in the cells differentiating on the collagen and sLe^x compared to control tissue culture plates, indicating enhanced myogenic progenitor formation. Furthermore expression of myogenic regulatory factors, Myf5 and Myogenin was significantly higher on both surfaces, indicating enhanced myoblast/myotube formation. Expression of the final differentiation marker MHC3 was also significantly higher for the matrix-cultured compared to the control.
Cardiac muscle: It was observed that a greater number of cells differentiated into cardiac muscle on the sLe^x surface, as judged by the number of beating foci. When compared to the control surface, the beating of the cells on the sLe^x surface was more synchronized. Expression of the cardiomyogenic genes GATA4 and Nkx2.5 and the cardiac muscle-specific peptide, ANF, was significantly higher on the sLe^x surface, indicating enhanced cardiomyogenesis.

Conclusions: These results demonstrate that the collagen matrix enhanced skeletal myogenesis, indicated by increased numbers of multinucleated myotubes and upregulation of skeletal muscle markers. The sLe^x – modification of the collagen matrix enhanced cardiomyogenesis in mouse ES cells, shown by increased beating and upregulation of cardiogenic genes. Thus we have identified biomaterials that could serve a two-fold purpose of providing an injectable substrate for delivery of stem cells and enhancing the progenitor population of both cardiac and skeletal muscles.

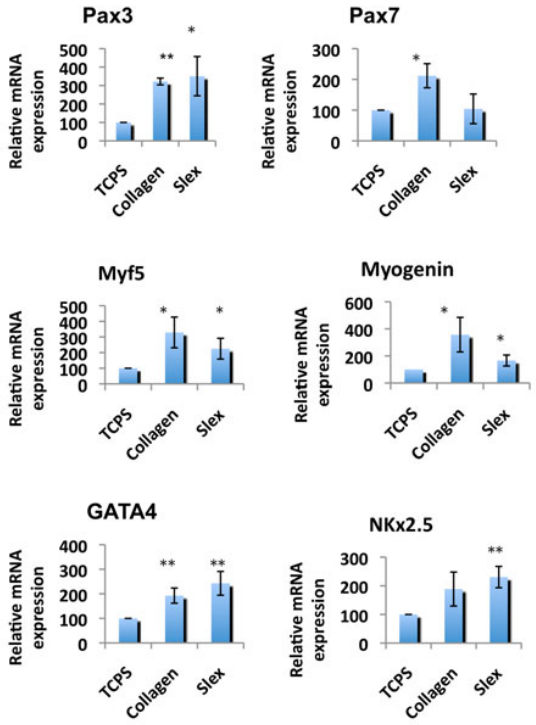

Figure 1- Q-PCR analysis of myogenic transcription factors on day 15 of mouse embryonic stem cell differentiation (n=6). * $p<0.05$ and ** $p<0.005$, significant difference between the matrices and the control tissue culture polystyrene plates.

References:
1. Suuronen EJ. FASEB. 2009;5:1447-58.
2. Kennedy KA. BMC Biol. 2009: 7:67.