Bioengineered skeletal muscle for defect replacement in a rodent surgical injury model

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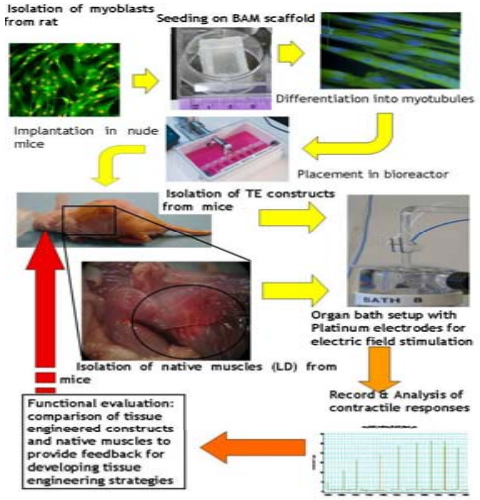
Introduction

The inability to engineer clinically relevant functional muscle tissue *in vitro* remains a major obstacle to the development of successful procedures for skeletal muscle (SKM) replacement *in vivo*. **The over-riding goal of our research program is to develop skeletal muscle biomimetics that can eventually be used clinically for restoration of muscle function** *in vivo*. Establishment of relevant pre-clinical animal models is a prerequisite for SKM tissue engineering and we are currently pursuing a variety of avenues in this regard. This report reviews a series of recent studies designed to examine the rodent latissimus dorsi (LD) as a model system for testing **functional** replacement of native SKM with tissue engineered SKM constructs. The rodent LD model offers several advantages for this purpose in that it is a readily accessible, thin sheet of SKM composed of mixed fiber types with numerous existing clinical indications.

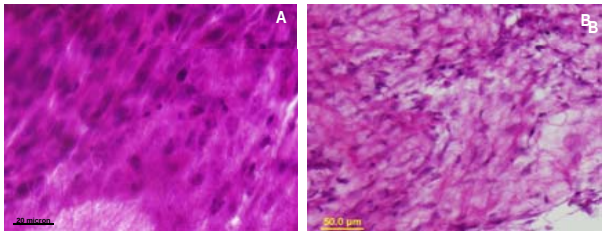
Methods

Native LD and Engineered skeletal muscle construct (TESKM) were carefully isolated from 10 month old male C57 BL mice and transferred to Krebs-Ringer buffer solution in a 15 ml Radnotti organ bath continuously bubbled with 95% O2 and 5 % CO2 at 25^0 C. Electrical field stimulation (20 Volt at electrodes, 0.2 ms square pulse) was applied to the muscle using parallel platinum electrodes. Optimal length was identified based on twitch response (0.2 ms pulse width at 20 volts); maximal isometric contractile force was measured at optimal length with a 1200 ms train of 0.2 ms pulses at different frequencies (10-150 Hz), normalized to cross section area and expressed as Specific Force (mN/mm2). Porcine bladder acellular matrix (BAM) was prepared as described (Moon et al., 2008) and rat muscle precursor cells were seeded and preconditioned in a bioreactor (Moon et al., 2008) for one week and implanted in nu/nu mice onto LD. The size of the engineered construct was 15 mm X 3mm X 0.35 mm; the latter dimension represents merely 1/10 of previously engineered constructs and is similar to native mouse LD. Similarly aligned polycaprolactone (PCL)/collagen scaffolds (Choi et al., 2008) ware also seeded, preconditioned in bioreactor and implanted in nude mice. In addition to this group, we created a muscle defect model by removing 50% of the area of native LD and replaced that with TESKM. Both the engineered construct and the contralateral native LD were explanted one month after implantation and functional properties were evaluated.



Results

Evaluation of tissue organization *in vitro* **on PCL/Collagen and BAM scaffolds seeded with myoblasts**



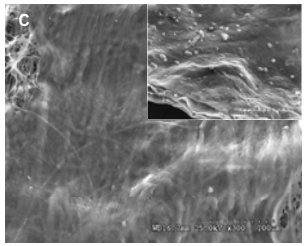


Figure 2. Tissue organization *in vitro***.** H&E section taken parallel to the seeded surface of a PCL/collagen aligned (A) and BAM scaffold (B) seeded with myoblasts. demonstrate the myotube formation on the surface of the scaffold, (C) SEM of myoblast seeded BAM scaffold shows the presence of myoblasts covering the surface of the scaffolds

Characterization of muscle tissue engineered (PCL/Collagen scaffold) *in vivo* **onlay model on LD**

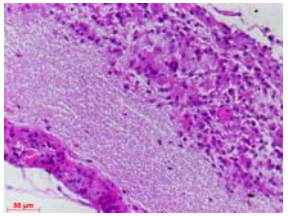


Figure 3. H&E staining of bioengineered skeletal muscle Aligned PCL/Collagen scaffolds with rat primary cells-1 week bioreactor explanted after 1 month in a Nude mice on LD muscle shows tissue formation (200 x/ CS).

MPC-seeded aligned PCL/collagen constructs were retrieved following placement as an onlay to the LD muscle. Two of these implants were retrieved one month after implantation, and two others were retrieved three months after implantation. In all four cases there were no observable contractile responses using our standard organ bath procedures. While there was indeed evidence for tissue formation, clearly, there was still a significant amount of scaffold material remaining, thus explaining the lack of contractility observed *in vitro*.

Comparison of tetanic response from retrieved tissue engineered construct (BAM scaffold as onlay model) and native muscle

Our initial experiment revealed that the tissue engineered SKM construct (BAM seeded with rat myoblasts – 1 week bioreactor) explanted after one month implantation on top of the mice LD showed a maximal isometric tetanic response of 42.9 mN/mm2 which was 26 % of that of native LD isolated from contra lateral side.

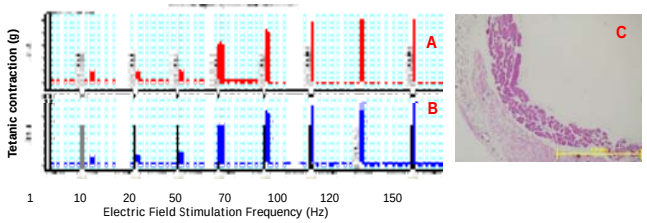
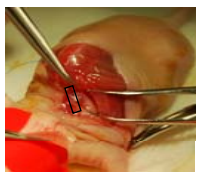
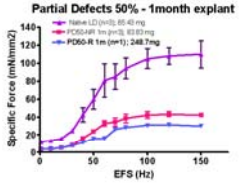


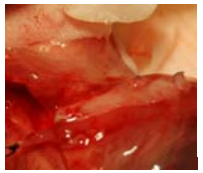
Figure 4. Comparison of tetanic response from retrieved BAM tissue engineered construct as an overlay model on LD and native LD. (A) Native muscle (B) TE-SKM construct retrieved 1 month after implantation in nude mice. (C) H&E of TE-SKM

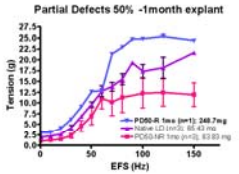
Functional Characterization of repair of muscle defect using BAM TESKM in an *in vivo* **model**

Figure 5. A surgical defect of approximately 50% of the area of the normal LD muscle was created (A- Box indicate the created defect) and replaced with a seeded BAM TESKM construct. The construct was explanted (B) after one month and physiological response was evaluated with native contralateral LD as well as non replaced 50% defect only controls. The figure shows isometric tetanic contraction expressed as specific force (C) and as total tension generated (grams) (D).









Conclusions

Our previous studies on larger constructs seeded with a similar number of MPCs yielded contractile responses with a specific force lower than that observed in this initial experiment. While further investigation is clearly required, these initial observations indicate that BAM based bioengineered SKM constructs, of dimensions similar to the rodent LD muscle they would replace, can generate approximately 26% of the normal isometric tetanic specific force observed in native muscles. To our knowledge, this is the largest degree of specific force yet recorded for engineered SKM on a decellularized matrix. Similarly in our defect model we were able to observe force generated by injured muscle repaired by TESKM to be that in the similar ranges of native muscle. However when normalized as the specific force of the repaired muscle, it was lower due to the higher mass of the TESKM construct. In short, the LD appears to be an attractive model for further studies of functional SKM replacement with engineered SKM constructs, providing additional evidence for the potential clinical utility of this approach.

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