Functional repair of skeletal muscle defects using tissue engineered skeletal muscle created from myoblasts seeded on bladder acellular matrix and preconditioned in a bioreactor

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Introduction: There are still no effective clinical treatments for the physiological deficits that result from traumatic injury, tumor excision, or other degenerative diseases/disorders of skeletal muscle (SKM). However, regenerative medicine/tissue engineering technologies have great potential for functional repair, reconstruction and replacement of damaged SKM. One such approach consists of developing clinically relevant functional muscle tissues *in vitro*, for subsequent implantation *in vivo*. This strategy consists of seeding muscle progenitor cells on scaffolds and preconditioning them in a bioreactor to enhance tissue maturation and function both in vitro and in vivo. The overall goal is to develop skeletal muscle biomimetics that can be used clinically for restoration of muscle function *in vivo.* Our initial studies have recently shown the potential utility of this approach[(1)]. The goal of the present investigation was to further extend these observations to determine the ability of tissue engineered skeletal muscle (TE-SKM) constructs to restore function when implanted in the latissimus dorsi of rat to correct a surgically created defect.

Methods: TE-SKM was developed by seeding rat myoblasts on BAM and preconditioned in a bioreactor as described (1). Cell seeded constructs were analyzed by H&E and SEM. Model for the muscle injury was developed by excising ≈50% surface area of the LD muscle in a nu/nu mice and repaired by suturing TE-SKM constructs at the excised sites. Thickness of TE-SKM was 0.35 mm which is merely 1/10 of previously engineered constructs and is similar to native mouse LD. Both the engineered construct and the contralateral native LD were explanted at either at one month or two months after implantation and transferred to Krebs-Ringer buffer solution in a 15 ml Radnotti organ at 28 0C. Maximal isometric contractile force was measured at optimal length with a 1200 ms train of 0.2 ms pulses, 20 V at different frequencies (1-150 Hz).

Results: Skeletal muscle myoblasts seeded on the BAM attached, differentiated to form myotubes and covered the entire area of the scaffold.

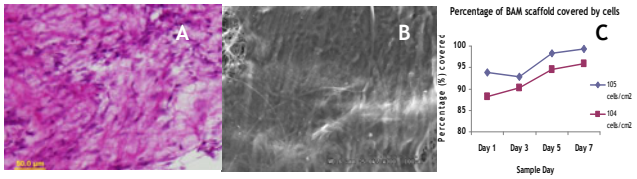

Figure 1. Tissue organization *in vitro*. H&E section taken parallel to the seeded surface of a (A) BAM scaffold seeded with skeletal muscle myoblasts demonstrate myotube formation on the surface of the scaffold, (B) SEM of TE-SKM shows the presence of myoblasts covering the surface of the scaffolds (C) quantification of growth of cells on BAM scaffold at two seeding densities.

Functional characterization of LD injury repair model

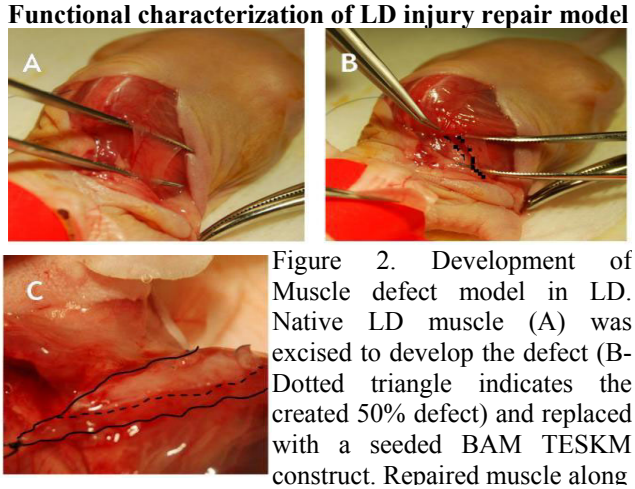

Figure 2. Development of Muscle defect model in LD. Native LD muscle (A) was excised to develop the defect (B- Dotted triangle indicates the created 50% defect) and replaced with a seeded BAM TESKM construct. Repaired muscle along with the construct was explanted after one month (C) (dotted line -construct, full line shows LD)

Following implantation, TESKM constructs generated higher force than un repaired group (12.6± 4.1 g) both at 1 month (16.88± 7.1 g) and 2 months (22.34± 5.6 g , $p<0.05$), the latter being ≈73% of that observed in native LD (30.67± 4.4g). A similar trend was observed when contra-ctile responses were expressed as specific force.

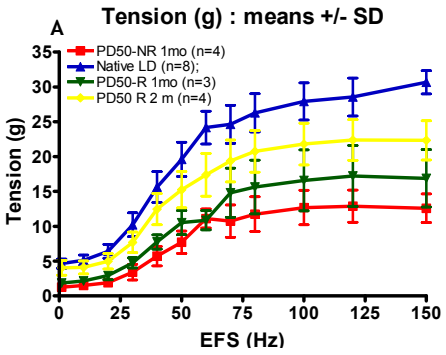

Figure 3 Isometric force tetanic force in relation to EFS frequency expressed as (A) absolute tension generated.

Conclusions: While further studies are clearly required, these initial observations indicate the applicability of this approach, specifically illustrating that following surgical removal of ≈50% of the LD muscle, implanted TE-SKM can recover ≈75% of the isometric tetanic force observed in native muscles within 2 months. In summary, the rodent LD defect model appears to be relevant for further proof of concept studies of the utility of TE-SKM for functional replacement of muscle defects.

References:(1)Moon, Tissue Eng 2008 Apr;14(4):473-82