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An acellular biologic scaffold does not regenerate appreciable *de novo* muscle tissue in rat models of volumetric muscle loss injury

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ABSTRACT

Extracellular matrix (ECM) derived scaffolds continue to be investigated for the treatment of volumetric muscle loss (VML) injuries. Clinically, ECM scaffolds have been used for lower extremity VML repair; in particular, MatriStem™, a porcine urinary bladder matrix (UBM), has shown improved functional outcomes and vascularization, but limited myogenesis. However, efficacy of the scaffold for the repair of traumatic muscle injuries has not been examined systematically. In this study, we demonstrate that the porcine UBM scaffold when used to repair a rodent gastrocnemius musculotendinous junction (MTJ) and tibialis anterior (TA) VML injury does not support muscle tissue regeneration. In the MTJ model, the scaffold was completely resorbed without tissue remodeling, suggesting that the scaffold may not be suitable for the clinical repair of muscle-tendon injuries. In the TA VML injury, the scaffold remodeled into a fibrotic tissue and showed functional improvement, but not due to muscle fiber regeneration. The inclusion of physical rehabilitation also did not improve functional response or tissue remodeling. We conclude that the porcine UBM scaffold when used to treat VML injuries may hasten the functional recovery through the mechanism of scaffold mediated functional fibrosis. Thus for appreciable muscle regeneration, repair strategies that incorporate myogenic cells, vasculogenic accelerant and a myoconductive scaffold need to be developed.

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1. Introduction

Severe musculoskeletal trauma often manifests a volumetric loss of skeletal muscle in civilian and military populations. The high prevalence of volumetric muscle loss (VML) among battlefield injuries has stimulated research efforts aimed at regenerating skeletal muscle *de novo* using regenerative medicine and tissue engineering approaches. These efforts are warranted since VML leaves a void and the remaining muscle alone cannot orchestrate the cascade of events that lead to successful regeneration typically seen in other recoverable muscle injuries [1,2].

A variety of tissue engineering strategies are under development for the repair of VML [3–15]. Among these approaches, decellularized extracellular matrix (ECM) scaffolds have translated to the clinic [16,17] and form the foundation for other cellular approaches as well [6,18]. The widespread clinical use of ECM scaffolds is primarily based on their inherent ability to provide a bio-inductive platform that supports endogenous cell recruitment, proliferation, and differentiation, ultimately improving healing as seen in an array of different types of tissue [19–28]. Additionally, ECM scaffolds are reported to support vascularization and induce macrophage polarization from a pro-inflammatory to a pro-remodeling response [29–32]. Favoring the use of these scaffolds for VML repair is the commercial availability of ECM derived scaffolds and their clinical use in other indications.

ECM scaffolds derived from different sources and species have been investigated extensively in preclinical animal models of VML [6,8–11,17,33–35]. Although, these scaffolds have improved muscle function in most studies [6,9,15,33,36–38]; scaffold mediated *de novo* muscle tissue regeneration has been limited and restricted to regions in proximity to the injured muscle [3,5,6,8,9,15,29,30,33,35,39–41]. However, studies that are contrary to these outcomes have also been reported. In a canine musculotendinous injury model, ECM scaffold implantation was shown to almost completely regenerate the lost musculature [33]. But, when used to repair a complex canine muscle-tendon injury [34] and a full thickness rodent abdominal wall defect [35], the presumably adequately processed ECM scaffold did not promote any

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recognizable muscle tissue regeneration.

On the basis of the positive results of the aforementioned pre-clinical animal studies, surgeons have used commercially available porcine derived small intestinal sub-mucosa (SIS Restore™, Cook Biotech, West Lafayette, IN, USA) and urinary bladder matrix (UBM MatriStem™, ACell, Baltimore, MD, USA) to repair lower extremity VML in a small cohort of patients [16,17,42]. Two of these studies have used the porcine UBM scaffold and reported improved functional outcomes, limited myogenesis, and vascularization in biopsy samples taken six months post-operatively [17].

Porcine UBM, MatriStem™, has been used clinically for the surgical reinforcement of soft tissue where weakness exits [43] and the general management of wounds [44–46] – the indications for which the scaffold has been approved by the U.S Food and Drug Administration (FDA). Although, the scaffold has previously been used for VML repair [17,42], there remains a need to establish evidence that will support further clinical use of the porcine UBM scaffold for the repair of traumatic muscle injuries. The overarching objective of this study is to investigate the efficacy of the acellular biologic scaffold as a tissue engineered strategy for the repair of traumatic muscle injuries. The primary objectives of the study are [1] to quantify the *in vivo* functional properties of injured muscle repaired with the porcine UBM scaffold, and [2] to characterize the histomorphological response of the remodeled tissue in rodent muscle injury models. An allied objective is to examine the functional and tissue remodeling benefit from physical rehabilitation in the injured muscle repaired with the scaffold.

2. Materials and methods

2.1. Experimental design

A musculotendinous junction (MTJ) or volumetric muscle loss (VML) injury was created in adult male Lewis rats and repaired with MatriStem™ (porcine UBM). For the MTJ injury, the defect created either received no repair or was repaired with a six-layer MatriStem™ Surgical Matrix PSMX. The rats from each treatment group were assigned to one of three time points: two, four, or eight weeks. For the VML injury, in an initial study, the defect created received no repair, was repaired with autograft tissue, or with a three - layer MatriStem™ Surgical Matrix PSM. The rats from each treatment group were assigned to one of three time points: two, eight, or sixteen weeks. The VML injury was also repaired with a syngeneic muscle derived ECM scaffold (m-ECM) decellularized using a previously established method [3,6] and was assigned only to the two week time point. At all time-points, the tissue was collected for histological analysis. *In vivo* function testing was done at four weeks post-MTJ injury, and, at eight and sixteen weeks' post - VML injury, followed by tissue harvest. In the case of VML injury, upon finding that the porcine UBM scaffold partially restored the functional capacity of the muscle at eight weeks; a second study was performed to determine if increased activity by way of physical rehabilitation during an eight week period would further improve function. In this study, the porcine UBM repaired rats were given free access to running wheels beginning at one week or four weeks post-injury, at which time *in vivo* muscle function testing and tissue harvest was done.

2.2. Animals

This study was conducted in compliance with the Animal Welfare Act, the Implementing Animal Welfare Regulations, and in accordance with the principles of the Guide for the Care and Use of Laboratory Animals. All procedures were approved by the IACUC at the U.S. Army Institute of Surgical Research. Rats were housed in a vivarium accredited by the Association for Assessment and Accreditation of Laboratory Animal Care International.

2.3. Injury models

2.3.1. Gastrocnemius musculotendinous junction (MTJ) injury

Forty eight adult male Lewis rats (3–4 months old; 325–350 g; Harlan Laboratories, IN, USA) were randomly divided into two groups, no-repair and porcine UBM repair, and three time points (n = 8/group/time point). All surgical procedures were carried out under anesthesia with continuous inhalation of isoflurane (2–3%). The animal was laid in the prone position and the foot taped in the neutral position (90°) to the surgical bed. Using aseptic technique, a longitudinal incision (~40 mm) was made to expose the Achilles tendon and biceps femoris muscle. The biceps femoris was then lacerated to expose the gastrocnemius muscle. The gastrocnemius muscle was separated from the underlying plantaris and soleus with blunt dissection. Distal third of the gastrocnemius muscle (~10 mm) and proximal third of the Achilles tendon (~3 mm) was excised. The resected tissue was replaced with size matched six - layer configuration porcine UBM. A six - layer porcine UBM was used as the gastrocnemius muscle is a load bearing muscle. After hydration in sterile saline for twenty minutes, the scaffold was sutured to the muscle and tendon, respectively, using a combination of mattress and simple suture configurations with Prolene suture (6-0). The biceps femoris and skin were closed using Vicryl and Prolene interrupted sutures (6–0), respectively. Thereafter, the animals were allowed free cage activity. At four weeks post-injury, the rats underwent *in vivo* muscle function testing.

2.3.2. Tibialis Anterior (TA) Volumetric Muscle Loss (VML) injury

Seventy two adult male Lewis rats (3–4 months old; 325–350 g; Harlan Laboratories, Indianapolis, IN, USA) were randomly divided into three groups, no-repair, autograft, and porcine UBM repair, and three time points (n = 8/group/time point). The autograft repair is included as a surrogate for muscle transfer, which is the repair strategy currently being used clinically for the treatment of VML injuries. Additionally, eight adult male Lewis rats were assigned to VML injury with m-ECM repair (two week time point). The surgical procedure for creating VML in the rat TA muscle was performed as described previously [6,7]. Briefly, using aseptic technique, a surgical defect (~10 × 7 × 3 mm) was created in the middle third of the TA muscle using a scalpel. The excised defect weighed approximated ~20% of the estimated TA muscle weight using a regression equation based on the rat's body weight at the time of surgery, as reported previously [47]. For the autograft repair, the VML defect was repaired by orthotopically placing the piece of TA muscle excised for creating the VML defect. For the porcine UBM repair, the excised muscle was replaced with size matched three - layer configuration porcine UBM. A three - layer porcine UBM was used as the TA muscle is a non-load bearing muscle. Prior to placement in the defect, the scaffold was hydrated for twenty minutes in sterile saline. Muscle derived scaffold (m-ECM) did not require hydration and was directly placed in the defect area. All scaffolds were sutured to the remaining TA muscle at the corners and the margins of the implant using prolene suture (6–0). These sutures also served as markers of the defect scaffold interface. At eight and sixteen weeks post-injury, the rats underwent *in vivo* function testing.

2.4. Physical rehabilitation with voluntary wheel running

A TA VML injury was created in sixteen adult male Lewis rats (3–4 months old; 325–350 g; Harlan Laboratories, IN, USA) and repaired using three-layer porcine UBM as previously described.